The Relations between Marine Animal and Vegetable Life.

By

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Contents.

Introduction.

Some three years ago, it was suggested to me by Prof. Ray Lankester, that an investigation into the conditions of life in marine Aquaria, especially with reference to the cycle of changes undergone by the nitrogenous matter excreted into the water, and also as to the effects of aeration, might afford results of both theoretical and practical importance. The work I then happened to be engaged upon, concerning the »Effects of Environment on the Development of Echinoderm Larvæ«[1], bore very closely on these questions, but I have not had an opportunity to continue these experiments, and extend them in the particular direction suggested by Prof. Lankester, until the present time.

The problem of the maintenance of marine aquaria in a state of equilibrium by the introduction of various forms of plant life, to remove the carbonic acid and nitrogenous matter excreted by the animals, is no new one. Such an aquarium on a very small scale appears to have been first successfully maintained by Dr. Johnston[2], as early as the year 1842. Subsequently Warington[3], by the introduction of healthy pieces of various algæ, as the Ulvæ, Enteromorphæ and Cladophoræ, which had carefully been left in contact with the nodules of flint or chalk from which they were growing, found it possible to maintain a state of equilibrium between the animal and vegetable life in a small tank for months, or even years. It was of course necessary to add a little distilled water from time to time, to counteract the effects of evaporation, but otherwise no renewal of the sea-water was required. Almost simultaneously with Warington's experiments, similar attempts at maintaining marine aquaria were made by P. H. Gosse[4]. Red weeds, especially *Chondrus crispus*, *Iridea edulis* and the Delesseriæ, were found by him to be more favourable than the green weeds, but he did not at first find it possible to maintain his aquarium for more than a month or two at a time without the introduction of fresh supplies of sea-water. Subsequently however, by the substitution of green weeds for red, he

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[1] Phil. Trans. 1895. B. pag. 577.

[2] History of British Sponges and Lithophytes. 1842.

[3] Gardeners Botanical Magazine and Garden Companion. Jan. 1852. also, Ann. Mag. N. H. (2) Vol. 12 1853 pag. 319 and Vol. 14 1854 pag. 366.

[4] Ann. Mag. N. H. (2) Vol. 10 1852 pag. 263.

was able to maintain an aquarium with the sea-water unchanged for months [1].

An inquiry into the conditions and inter-relations of marine animal and vegetable life has thus a very practical bearing, but, quite apart from this, it is of interest to know something of the processes taking place on a vast scale in the open sea. What are the most effective agencies in the removal of this nitrogenous waste material excreted by the animals, and under what conditions do they act? Are they chiefly or almost wholly of a vegetable nature, or are other important factors at work? Such are the questions it is here attempted to answer.

Method of Experiment.

In the present inquiry it was found necessary to attack the problem from a triple standpoint, from the chemical, the physiological, and the bacteriological. The physiological part of the research consisted in the continuation of the method described in the above mentioned paper. In this method, the larvæ of the sea-urchin *Strongylocentrotus lividus*, which are readily obtained from artificial fertilisations, are grown in the various specimens of water, obtained under various conditions, and then after eight days' growth are killed and preserved, and measured in series of fifty under the microscope. From the mean size of the larvæ obtained under these various conditions, as compared with that of the larvæ obtained on growth under normal conditions in ordinary tank water, it was possible to deduce conclusions as to the purity or otherwise of the water under examination. In every case two measurements of each larva were made, namely of the body length, and of the aboral arm length. These latter were then always calculated as percentages on the former. The mean values obtained for these measurements are given in the table at the end of the paper. They are given in scale units of the micrometer eyepiece. If it be desired to reduce them to millimetres, they must be divided by 152.3. In the table it will be seen that altogether 140 series of measurements, each comprising fifty larvæ, were made. The few experiments marked with an asterisk were made upon the larvæ of *Sphærechinus* or *Echinus*, and not those of *Strongylocentrotus*. In addition to measuring the size of the larvæ, the numbers of ova used in each case, and the number of blastulæ

[1] The Aquarium 1854 pag. 13.

which had developed therefrom after twenty-four hours' growth were always carefully counted. This counting was done very simply by thoroughly stirring up the jars of water containing the ova and blastulæ, and then withdrawing 3 cc. of the water with a pipette, and running it into a small glass cell, into which a single drop of saturated corrosive sublimate had previously been introduced. A cover slip was then slid on the top of the cell, and after waiting a few minutes to allow the ova and blastulæ to settle, it was fixed in a mechanical stage, and its whole area worked carefully through under the field of the microscope. By making these observations after twenty-four hours' growth, one can determine at the same time both the number of blastulæ and the number of ova which have remained unfertilised, or have not developed normally to the blastula stage. In each case two separate countings were made, and a mean taken. If these two values differed at all largely, a third counting was made. In the table at the end of the paper are given in each case the number of ova present in 10 cc., the percentage numbers of these ova which had developed to the blastula stage after twenty-four hours growth, and the number of plutei formed after eight days growth. These latter numbers are also the means of two separate countings, made in a similar manner to those of the ova and blastulæ. For further details as to the method of making and carrying out these artificial fertilisations of Echinoid ova, the paper mentioned above may be consulted.

The chemical side of the investigation consisted in making determinations of the free and the organic or so-called albuminoid ammonia present in the various specimens of water. The method adopted was that of Wanklyn and Chapman[1]. It consisted in distilling half a litre of the water under examination, and collecting the distillate in tall cylindrical glasses in separate volumes of 50 or 100 cc. To each 50 cc. of distillate, 2 cc. of Nessler's reagent, a strongly alkaline solution of mercuric iodide in potassium iodide, is added, and the brown colouration thereby produced determined in terms of a standard solution of ammonium chloride, to which the Nessler reagent has also been added. This comparison was made by means of a colorimeter. The tube containing the distillate was allowed to stand on a glass plate, and close by it a similar but graduated tube, in which, by means of a tubulure near the bottom, the height of the

[1] Water Analysis. 10th Ed. pag. 33.

standard solution could be varied at will. White light was reflected up the tubes by means of a porcelain plate placed at an appropriate angle underneath the glass plate, and the two tubes were viewed from above through a mask with two circular holes in it. The amount of ammonia present could thus be determined far more readily and more accurately than by the method adopted by WANKLYN and CHAPMAN, in which separate standard solutions of various strengths have to be made up, until one of the same degree of colouration as the solution under examination is hit upon. After 200 cc. have been distilled off the 500 cc. of water, whereby all the free ammonia present is removed, 50 cc. of a strongly alkaline solution of potassium permanganate solution is added, and a further 150 cc. distilled off. This distillate contains the so-called albuminoid or organic ammonia present in the water. It was found by WANKLYN and CHAPMAN[1] that most organic substances such as egg albumin, gelatin and amylamine compounds, yield, on distillation with alkaline permanganate solution, the whole or a large part of the nitrogen they contain, in the form of ammonia. This method therefore gives one a fair criterion of the amount of organic impurity contained in the water. The amount of free ammonia present at the same time also affords confirmatory evidence as to the degree of contamination present. Nevertheless, the method is not to be regarded as a very exact one, or as affording an absolute measure of the degree of organic impurity. Thus, for instance, it was found that in one case when a specimen of water was distilled 2.3 times more slowly than usual, the amounts both of free and organic ammonia were increased by some 10%. At the same time the method is probably the simplest and best one available for estimating the quality of a water. It was not thought worth while to make other determinations, as of the amount of oxygen used up on the addition of excess of dilute permanganate solution, as the results thereby obtained add but little to those yielded by the ammonia process.

The bacteriological side of the investigation consisted merely in making gelatin plate cultures of the various specimens of water, and counting the number of colonies present after the cultures had been kept 24 and 48 hours in a moist chamber.

This research naturally divides itself into two parts: the purification of water by vegetable life, and the fouling of water by animal life. The former will be dealt with first.

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[1] ibid. pag. 182.

Part 1. The Purification of Water by Vegetable Life.

The Effects of Algæ on the Purification of Water.

a. The Effects of Ulva latissima.

As the observers above mentioned found that equilibrium could be obtained in marine aquaria by the introduction of vegetable life in the form of growing algæ, it was natural that the purifying effects of plant life upon sea-water should first be studied. A series of experiments was therefore made upon the influence of *Ulva latissima*, which has been found so favourable by other investigators. In order to determine the effects of the alga upon the ammonia present in the water, a piece of the *Ulva*, attached to a stone, was placed in a covered jar holding about $3^1/_2$ litres of sea-water, and this was exposed to diffuse daylight at a temperature of about 21° C. The *Ulva* was present in the proportion of about 12 square centimetres per litre of water, and when any water was removed from the jar for analysis, a corresponding quantity of the weed was removed at the same time. Analyses of the water from time to time gave the following results.

	Ammonia present in milligrams per litre			Ammonia present in milligrams per litre	
	Free	Organic		Free	Organic
Original water	.223	.134	After 10 days	.012	.170
After 2 days	.085	.163	- 16 -	.007	.214
- 4 -	.046	.150	- 28 -	(.141)	(.567)

From this table it will be seen that the free ammonia present is very rapidly diminished, it being decreased by 62 % after two days. The organic ammonia on the other hand slowly increases in amount. When the proportionate amount of *Ulva* present is very considerable, even the free ammonia may be increased. Thus in the last observation cited in the above table, a volume of only 300 cc. of the water was left in contact with 24 sq. cm. of *Ulva* for twelve days.

In a parallel experiment to the above, in which some of the same sea-water was exposed to the action of sunlight, instead of diffuse light, but to which only half the quantity of *Ulva* was added, the following result was obtained.

	Free NH_3	Organic NH_3
Original water	.223	.134
After 2 days	.202	.194
- 4 -	.147	.239

In this instance we see that the exposure to direct sunlight had by no means a favourable effect on the action of the alga, as the free ammonia was diminished much more slowly, and the organic ammonia increased much more quickly in amount than before. Unfavourable results in experiments of this kind cannot, however, be considered of much importance, as they may be due merely to the alga undergoing an abnormal decomposition, due to the artificial conditions. Thus in the present case, the *Ulva* was to a large extent bleached by the action of the sunlight.

Judging from the rapidly purifying effect of *Ulva* upon the free ammonia present in the water, one might expect that water thus treated would have a beneficial effect upon animal life, as evidenced by the growth of Echinoid larvæ. Such seems to be actually the case, if precisely the best conditions as to the amount of *Ulva* used, and the time it has been allowed to act, be observed. Thus larvæ grown in a sample of the above mentioned water, after treatment with *Ulva* for four days, were found to be 14.4% larger than those grown in normal tank water (vide Exp. 50). Also larvæ grown in the water exposed to the action of *Ulva* in direct sunlight for four days, were 16.8% larger than the normal (Exp. 39). As will be shown later on however, this favourable effect must have been largely due to the direct effect of the sunlight on the water. In another experiment, No. 9, larvæ were allowed to grow in a specimen of water in which other larvæ, together with some *Ulva*, had previously been growing for ten days. They were found to be practically unaltered in size, they being .06% smaller. On the other hand in a parallel experiment, No. 10, in which larvæ were grown in a specimen of water in which other larvæ had been allowed to develop without the addition of *Ulva*, the size was diminished by 6.8%. In both these instances the original larvæ were of course filtered off from the water before the fresh fertilised ova were introduced.

A negative effect is, however, produced in some cases. Thus in Exp. 93, in which the water was exposed to the action of 12 sq.cm. of *Ulva* per litre for four days, whereby the free ammooia present was diminished by 46%, and the organic ammonia increased by 8%, the larvæ were diminished 1.0% in size.

In the experiments thus far described, the larvæ were allowed to grow in water previously exposed to the action of the alga. Another series was made, in which pieces of *Ulva* were added to the water in which the fertilised ova were developing, and in which therefore the larvæ were in direct relation to the alga throughout their growth. The results obtained are given in the table.

Number of Expt.	Amount of *Ulva* per litre.	% Larvæ formed	% Difference in Size of larvæ from normal	% Difference in amount of NH_3, from that present in normal experiment	
				free NH_3	organic NH_3
43	4.5 sq.cm.	100	+ 4.0	— 16.6	— 12.8
41	6 sq.cm.	72	— .4	— 9.2	+ 6.1
108	6 sq.cm.	99	— .7	—	—
24	6 sq.cm.	90	— 13.2	— 47.7	— 17.3
89	15 sq.cm.	69	— 1.6	— 54.3	— 15.1
2	60 sq.cm.	51	— 3.4	— 93.1	— 21.8

Here it will be seen that only in one case, Exp. 43, were the larvæ increased in size. In two others they were only very slightly affected, but in the other three distinctly reduced in size. With the exception of Exp. 24, the alga seems to have exerted a more and more unfavourable effect in proportion to the amount present in the water. Presumably in all experiments but the first in the table, the amount of *Ulva* used was too large. In the last two columns of the table are given the percentage differences in the amounts of free and organic ammonia in the water after eight days growth of the larvæ, as compared with the amounts present in parallel series of experiments, in which the larvæ were allowed to grow without the addition of any *Ulva*. From these figures it will be seen that in every case the amount of free ammonia was considerably reduced, this reduction being more or less proportionate to the amount of *Ulva* present. In four out of the five observations made the organic ammonia was also reduced considerably in amount. Contrary to the result previously obtained, it must be concluded therefore, that when the amount of organic ammonia present is fairly large, as is the case when larvæ are growing in the water, the alga has the power of partially removing it. In the light of these ammonia determinations, it seems all the more curious that the larvæ should have been as a rule diminished in size. It is possible however that the mere size

does not at all times and under all conditions afford a just criterion as to the effects of a particular water, whether favourable or otherwise, upon the growth of animal life in general. Thus in the third column of the above table are given the percentage numbers, on the ova used, of the larvæ which survived to eight days' growth. It will be seen that in all but the last experiment in the table, in which the amount of *Ulva* present was excessive, 69% and upwards of the ova developed into full grown larvæ. Now as a mean of the sixteen experiments made from time to time during the course of six months with larvæ growing in normal tank water under average conditions, it was found that only 66.6% of the ova used developed into full grown larvæ. As in the present series, excluding the last experiment, it is found that on an average 86% of the ova so develop, it follows that the addition of moderate amounts of *Ulva* has caused about 20% more of the ova to develop to maturity.

One or two other observations bearing on the effects of *Ulva* may here be cited. Thus in Exp. 67 a mollusc, *Cerithium vulgatum*, weighing 6.2 gm, and with about 8 sq.cm. of *Ulva* growing from its shell, was placed in two litres of water containing fertilised ova. The amount of *Ulva* was insufficient to absorb all the ammonia excreted by the mollusc, as after eight days the amounts of free and organic ammonia present were respectively 1.295 and .181 mgm. per litre, as compared with the .528 and .296 mgm. present in the case of larvæ grown under normal conditions. Nevertheless the larvæ grown in company with the mollusc were only .9% smaller than the normal. Again, in Exp. 109, a similar mollusc, weighing 5.6 gm., and with 15 sq.cm. of *Ulva* growing from its shell, was placed with the fertilised ova in $2^1/_2$ litres of water. The resulting larvæ were 3.6% smaller than the normal. In Exp. 110, however, in which the ova were allowed to develop with another mollusc of the same species, weighing 6.5 gm., but without any *Ulva* growing from its shell, the resulting larvæ were 12.6% smaller than the normal. The presence of the *Ulva* seems therefore to have made a difference of 9% in the size of the larvæ.

b. The Effects of Other Kinds of Algæ.

As has already been mentioned, Gosse was able to keep other algæ, such as certain red weeds, alive in his aquaria, and he at first thought them to be more favourable than the green weeds. Warington also succeeded perfectly in growing red weeds in his

aquaria[1], but be found that for this purpose it was necessary to diminish the light to which they were exposed by means of green or blue shades.

In the present series of observations, the red weeds did not appear to keep in such a healthy state as was the case with *Ulva*, and at the end of eight days the tips of the fronds were generally tinged with orange, a sure sign of decay. This was not the case in the following experiment however, in which a frond of *Gelidium*, 2 grams in weight, which was attached to a small piece of stone, was placed in a jar of $3^1/_2$ litres of water. Analyses of this water were made from time to time, the amount of weed being diminished proportionally to the water removed.

	Free NH_3	Organic NH_3
Original water	.314 mgm.	.139 mgm.
After 4 days	.421	.176
- 11 -	.223	.153
- 21 -	.171	.245
- 28 -	·069	.426

From these analyses it would seem that the red weed was able, after acting for some days, to slowly absorb some of the free ammonia present. It is possible, however, that this apparent absorption was due to the diatoms and algæ which had begun to multiply in the water on standing. The organic ammonia gradually increased in amount, as was the case with *Ulva*. After 21 days, the relative weight of the *Gelidium* was increased, it now being .8 gm. in only 700 cc. of water. It also began to show signs of decay, but, nevertheless, the free ammonia was absorbed with greater rapidity, whilst the organic ammonia more rapidly increased.

As in the case of *Ulva*, a few observations were made in which the water was exposed to the action of the alga previous to the introduction of the fertilised ova. They gave the following results.

Number of Expt.	Weight of alga per litre	% Larvæ formed	% Variation in Size of larvæ	% Variation in amount of	
				free HN_3	organic NH_3
94	1.0 gm. of *Gelidium corneum*	91	— 4.1	+ 110	+ 9
132	.6 gm. - -	87	± 0.0	+ 34	+ 27
95	2.0 gm. of *Dudresnaya purpurifera*	13	— 18.1	+ 25	+ 179
96	.7 gm. of *Dictyota*	92	— 6.8	+ 79	+ 40

[1] Ann. Mag. N. H. (2) Vol. 14. 1854 pag. 371.

In each of these experiments the alga was allowed to act for four days. In every case but one the water was rendered very harmful to the growth of the larvæ, as far as size was concerned, but in three out of the four cases the actual number of larvæ developing was considerably larger than the normal. Probably the increase in the amount of ammonia present, as determined after four days exposure to the action of the alga, is sufficient to account for the diminished size of the larvæ. The organic ammonia seems to exert the most harmful influence. Thus in Exp. 95, in which it was 179 % greater than that in the normal water, the larvæ were diminished by 18.1 %.

Experiments were also made in which the fertilised ova were allowed to develop in contact with the alga. The results obtained are given in the following table.

Number of Expt.	Weight of alga per litre	%Larvæ formed	% Variation in size of larvæ	% Variation in amount of	
				free NH_3	organic HN_3
54	.2 gm. *Gelidium corneum*	74	+ 4.1	+ 130	— 2
90	.8 gm. - -	21	— 4.1	+ 10	— 12
92	1.2 gm. *Dudresnaya purpurifera*	51	— 2.7	—	—
68	.13 gm. *Gelidium* + 6 sq.cm. *Ulva*	55	+ 3.6	+ 5	— 29
133	.3 gm. - with *Ulva* attached	79	— .4	+ 11	+ 50
42	.4 gm. - + 3 sq.cm. *Ulva*	99	— .9	+ 20	+ 113
91	.6 gm. *Dictyota*	23	— 3.1	—	—

In the three experiments made on the effects of red weed alone, the larvæ were increased in size in one case by 4.1 %, in spite of the fact that, as compared with the water in which larvæ had been grown under parallel conditions, but without any alga, there was a large increase in the free ammonia present. It is difficult to account for the larvæ in the next experiment, No. 90, being diminished 4.1 % in size, for the ammonia present in the water was altered but little. Probably in the one case the alga was in a healthy condition, and in the other not. In Exp. 92 the alga was obviously unhealthy, and by the end of the eight days it had almost completely changed from a pink to a green colour.

In that red weeds appear as a rule to increase the amount of free ammonia present in the water, as well as the organic, it was

thought possible that a combination of small quantities of green and red weeds together might have a favourable effect upon the growth of the larvæ. In Expts. 68, 133 and 12 are given the results obtained. Here it will be seen that in one case the larvæ have increased 3.6% in size, and in the other two slightly dimished. The percentage number of ova reaching the larval stage is, however, greater than the normal, and hence on the whole the effect appears favourable, in spite of the ammonia present having as a rule increased. In the last experiment in the table, made with the branching green weed *Dictyota*, the larvæ were 3.1% smaller than the normal, probably owing to the fact that the alga had got into an unhealthy condition towards the end of the experiment.

The observations on red weeds were not continued, because of the difficulty of getting them to live in good condition. In some cases, as in experiments 42, 90, 92 and 133, pieces of weed attached to stones were obtained, but this seemed to make no difference. In the case of *Ulva*, the alga appeared to remain equally healthy, at any rate for eight days, whether it was attached to a stone or not. Thus in Expts. 39, 11 and 42 pieces of the alga growing from stones, and in the other experiments torn off pieces, were used.

A comparison of the effects of green weeds such as *Ulva*, and of red weeds such as *Gelidium*, upon the purification of the water and the growth of larvæ, obviously speaks much in favour of the *Ulva*. Thus this alga rapidly removes the free ammonia present in the water, and probably under certain conditions may diminish the organic ammonia. Also it seems to keep in a healthy condition much more readily. Red weeds on the other hand, though they seem in some instances to exert a favourable influence on the growth of the larvæ, increase the ammonia present in the water, and are moreover exceedingly difficult to keep in a healthy condition.

The Effects of Diatomaceæ and minute Algæ.

a. The Effect of Filtration through Sand.

Filtration through sand is the method most frequently adopted for the purification of fresh water for drinking purposes. Until comparatively recently, this purifying action was looked upon as a mechanical one, but R. Koch has shown[1] that it must be attributed

[1] Zeit. Hyg. 14. Bd. pag. 395.

to the layer of slime or zoogloœa which gradually forms on the surface of the sand, as a deposit from the filtering water. Thus it is known that a fresh sand filter takes several days to attain its full filtering efficiency.

In order to try the effect of such filtration on the Aquarium tank water, a stream of water was allowed to run slowly, at a rate of about two litres per hour, through a layer of moderately coarse sand 14 cm. in depth, and of about 100 sq.cm. superficial area. The sand used in this experiment had been taken from one of the tanks in the Aquarium. The following was the change in the composition of the water.

	Free NH_3	Organic NH_3
Before filtration	.268 mgm.	.165 mgm.
After filtration	.017	.136

That is to say, by simple filtration through sand, no less than 94% of the free ammonia present in the water, and 18% of the organic ammonia had been removed! It seemed impossible that such purification could be due merely to mechanical filtration. That this was obviously not the case was proved by a microscopical examination of the sand. It was then found that every grain of sand was covered with a thin layer of structureless particles of alga, and here and there with diatoms. The removal of the ammonia must have been due to this vegetable agency. That this was so was confirmed by another experiment, made with some of the same sand, which had however been left stagnating in a jar of water for ten days, and which probably on this account did not act so efficiently. Thus on filtering water through a layer of this sand, 11 cm. in depth, 79% of the free, and 37% of the organic ammonia, were removed. Some of this sand was then heated to 70° C. with sea-water, by which means all the vegetable life present was killed. It was then washed several times, to remove as much of the dead vegetable debris as possible. On filtering water through a layer of this water sand, 7 cm. in depth, so far from the ammonia present in the water being diminished, it was found that the free ammonia had increased 18%, and the organic ammonia 272%, or nearly four fold.

With a view to making a more accurate estimate of the conditions of water purification by sand, an apparatus consisting of a large inverted tubulated bell-jar was set up. Over the tubulure of the bell-jar was placed a small porcelain sieve, and on the top of this a small inverted glass dish. The bell-jar was then filled to a depth

of 16 cm. with the sand under examination, and a stream of water allowed to flow in from above. The sieve and dish over the tubulure entirely prevented any sand from being carried away by the current of water. The depth of water when the bell-jar was full was 12 cm. The diameter of the jar was 15 cm., thus giving a superficial area of about 177 sq.cm. of sand. Through this apparatus, and another of exactly similar construction, currents of water were allowed to flow continuously for several weeks, the entering and outflowing water being analysed from time to time. The following results were obtained for filtration through fine sand, which had been kept stagnating in a small tank in the Aquarium for some weeks, and which was impregnated with but small quantities of diatoms and algæ.

Day of Expt.	Time of filtration of 1 litre	% Variation in amount of		Time of filtration of 1 litre	% Variation in amount of	
		free NH_3	organic NH_3		free NH_3	organic NH_3
1st	3′ 15″	— 56	— 12			
2nd	3′ 15″	— 60	— 10			
4th	2′ 40″	— 31	— 14			
8th	3′ 6″	— 58	— 21	7′ 45″	— 90	— 15
				50′ 0″	+ 221	+ 2
15th	3′ 50″	— 96	— 51	11′ 20″	— 90	— 34
				28′ 0″	— 85	— 24
				13h 0′	+ 215	— 7
25th	11′ 45″	— 92	— 12			
29th	4′ 0″	— 87	— 5	1h 20′	+ 221	+ 12
36th	9′ 30″	— 92	— 21	2′ 0″	(— 84)	(— 33)
42nd	6′ 20″	— 89	— 6			
43rd	8′ 45″	— 89	— 21			
44th	10′ 30″	— 87	— 20			
49th	17′ 0″	— 90	— 15			
54th	30′ 0″	— 91	— 14			
59th	47′ 0″	— 94	— 19			
66th				2h 50′	— 90	— 16
67th				2h 40′	(— 93)	(± 0)

On the left side of this table are given the results obtained when the water was allowed to flow through at its maximum rate, whilst in those given on the right side, the outflow tube was clamped in varying degrees. From this table it will be seen that during the first week of the experiment only about half of the free ammonia was removed from the water. At the end of the second week, however, as much as 96% was removed. The values for the organic ammonia removed show a most striking increase in amount from day to day, only 12% being removed on the first day of the experiment, and as much as 51% on the fifteenth. On the 22nd day of the experiment the current of water was by inadvertence stopped from running through the sand for twenty-four hours. This had a very harmful effect on the purifying capacity of the sand as far as the organic ammonia was concerned, but this capacity began to return somewhat after a few days. Also the maximum rate at which the water would flow through the sand was diminished some threefold. This was due to the thin layer of diatoms and algæ, which was gradually being formed on the top and in the interstices of the sand by deposition from the current of water, being partially killed and transformed into a more cohesive and homogeneous layer. Thus on the 26th day of the experiment, a number of small holes were made through this vegetable crust on the top of the sand by means of a needle, whereby the rate of flow of the water was increased threefold for the next few days. As these small holes became filled up however, the rate of flow gradually became almost as slow as before. Accordingly on the 36th day of the experiment, after collecting and analysing some of the outflowing water, the crust on the top of the sand, consisting of a layer about 2 mm. thick, was scraped away, and the water now ran through at the rate of 1 litre in 2 min., instead of 1 litre in 9½ min.; moreover, as may be seen from the figures in the right half of the table, the water when running through at this increased rate, and without the influence of the vegetable crust on the top of the sand, was if anything more purified than before. Thus though 8% less of the free ammonia disappeared, yet 12% more of the organic ammonia did so. It seems somewhat extraordinary that the removal of the vegetable crust on the top of the sand, which was imagined to be the chief cause of the purification, should have produced a favourable rather than an unfavourable effect. The continued purifying efficiency of the sand must have been partly due to the layer of diatoms and algæ which could be seen collecting

round the sides of the bell-jar, where of course the sand was exposed to daylight, but it would seem to have also been due to the action of bacteria or other organisms contained in the sand. Thus on the 42nd day, when the rate of filtration had again become considerably slower, and the purifying power of the filter somewhat less, the whole apparatus was covered up with black paper, so as to prevent any trace of light reaching the sand. It was kept in this condition till the termination of the experiment, 25 days later. During this time the rate of filtration gradually became slower and slower, till at last only about one litre per hour ran through. The purifying action of the filter if anything increased however, though the amount of organic ammonia removed never reached so high a level as when the filter was exposed to light. That this purification was due to other than chlorophyll containing organisms, would necessarily follow from the absolute exclusion of light. It was proved also by a microscopical examination of the top layer of sand, which showed that these were no longer present. A still further proof that these chlorophyll containing organisms took no part in the process is afforded by the series of experiments given in the right half of the above table. From these it may be seen that in each of the three instances where the rate of filtration was so diminished that a litre of water took fifty minutes or more to run through, the free ammonia in the water was increased some three- or fourfold, though the organic ammonia was but slightly affected. Now it may be seen in the above table, that with the filter excluded from light, water running through at the rate of a litre in 47 minutes had 94% of its free ammonia removed, and 19% of its organic ammonia; and that when on the 66th day, by clamping the outflow tube, the water was made to filter through at only a litre in 2 hrs. 50 min., the purification was still nearly as great. Even the water drawn off at about the same rate on the next day, immediately after the flow of water through the filter had been entirely stopped for ten hours, was just as purified in respect of its free ammonia, though none of its organic ammonia had been absorbed. In fact, it would seem that within certain limits, with the sand excluded from light, the slower the rate of filtration the greater is the efficiency of the filter. On the other hand, with sand impregnated with chlorophyll containing organisms, the quicker the rate of filtration, the better the purification. Thus in the experiment made on the 15th day, considerably less ammonia was removed when the rate of filtration was diminished from a litre in

3′ 50″ to a litre in 11′ 20″, and still less when decreased to a litre in 28′. It should be mentioned that, before collecting the samples used for these analyses, the water was allowed to filter at the same slow rate for several hours previously. With reference to fresh water sand filtration, KOCH has shown[1] that to obtain water containing less than 100 germs per cc., the rate of filtration must not be more than 100 mm. per hour. Calculated for the filter at present under discussion, this would correspond to a rate of 1 litre in 34′. The New River Company, on the other hand, filter their water[2] at a rate corresponding to 1 litre in 22′. These rates of filtration were however determined with reference to the removal of as many germs from the water as possible, and not to the most efficient removal of the ammonia.

Simultaneously with the above series of observations, another was made on the filtering capacities of a specimen of sand which had been dug up from the sea shore, at a depth of a metre beneath the surface. It could obviously therefore be uncontaminated with any vegetable growth of a chlorophyll containing nature. The results obtained with this sand are given in the accompanying table. As before, the layer of sand was 16 cm. thick, and of 177 sq.cm. superficial area.

Day of Expt.	Time of filtration of 1 litre	% Variation in amount of		Time of filtration of 1 litre	% Variation in amount of	
		free NH_3	organic NH_3		free NH_3	organic NH_3
1st	1′ 45″	— 12	— 6			
4th	1′ 50″	— 7	— 6			
8th	2′ 8″	— 23	— 12	2 hours	+ 33	— 2
15th	2′ 6″	— 75	— 37	2h 46′	+ 76	— 18
25th	7′ 15″	— 92	— 11			
29th	8′ 15″	— 87	— 6	2′ 40″	(— 89	— 2)
				2′ 35″	(— 53)	(— 9)

For the first few days we see that this sand had practically no ammonia-absorbing powers at all, the slightly diminished amounts present in the filtered water probably being due to the mechanical

[1] ibid.

[2] NOTTER and FIRTH, Theory and Practice of Hygiene 1896 p. 47.

removal of suspended organic matter. After a week's filtration however, the sand had become impregnated with enough vegetable growth to remove a quarter of the free and an eighth of the organic ammonia, whilst after a fortnight three quarters of the free and a third of the organic ammonia were absorbed. As in the previous case, the cessation of the current of water for twenty-four hours on the 22nd day of the experiment caused a great diminution in the absorption power of the sand for organic ammonia, though the free ammonia was removed more efficiently than before. The rate of filtration of the water was also diminished more than threefold. On the 29th day of the experiment, after collecting and analysing a specimen of the water, the top half centimetre of the sand was scraped away, and the outflowing water again analysed. It now filtered through three times more rapidly than before, but was just as efficiently purified of its free ammonia. Another layer of sand, two centimetres in depth, was then removed, and the water again analysed. Its purifying action on the free ammonia was now considerably less than before, but this may have partly been due to the greater rapidity of the filtration. As in the previous series of observations, this continued purifying capacity of the sand could scarcely have been entirely due to the layer of diatoms and algæ with which the sand exposed to the light at the sides of the bell-jar was impregnated. Bacteria or other organisms deposited in the sand itself must have effected some of it. As before, the effect of diminishing the rate of filtration was also determined. In that, owing to the presence of much smaller quantities of vegetable growth, the purifying capacity of this filter was much less, so one would expect, on a slowing of the current, that the increase in the amount of ammonia added to the water by the decomposition taking place in the vegetable growth would also be smaller. Such is actually the case. Thus on the eighth day of the experiment, with a very slow rate of filtration, the free ammonia was increased by only 33%; and on the 15th day, by only 76%, instead of the 200% and upwards observed in the former case.

In addition to testing the purification of the water by chemical means, it was tested physiologically by observing its effects on the growth of larvæ. The following were the results obtained.

Number of Expt.	Nature of sand used	Time of filtration of 1 litre	% Variation in amount of free NH_3	% Variation in amount of organic NH_3	% Larvæ formed	% Variation in Size of larvæ.
55	Coarse Aquarium sand	30′ 0″	— 94	— 18	100	+ 6.2
63	Same sand kept stagnating 10 days	22′ 0″	— 79	— 37	100	+ 5.1
64	Same sand previously heated to 70° C.	30′ 0″	+ 18	+ 272	80	(+ 1.3)
65	Fine Aquarium sand	20′ 0″	— 13	— 30	98	+ 5.4
99	Stagnant Aquarium sand	3′ 15″	— 56	— 10	54	+ .6
100	Surface shore sand	3′ 30″	+ 185	— 4	8	+ 4.7
101	Shore sand 1 metre below surface .	1′ 45″	— 12	— 6	58	(+ 1.8)
123	Aquarium sand	4′ 0″	— 87	— 5	72	+ .7
124	Same sand	1h 20′	+ 358	+ 12	88	+ 2.0
125	Shore sand 1 metre below surface .	8′ 15″	— 87	— 6	100	+ 8.5
126	Same sand with top crust removed	2′ 40″	— 89	— 6	88	+ 4.2

In every experiment given in this table it will be seen that the filtration of the water through sand had a favourable influence on the growth of the larvæ, and also that in the great majority of cases the percentage of ova which developed to eight days Plutei was greater than the normal (i. e. 66.6%). To discuss individual experiments, we see that in the first one given in the table, in which the water was filtered through coarse sand taken from one of the tanks of the Aquarium, whereby nearly all the free ammonia was removed, the larvæ were 6.2% larger than the normal. Larvæ grown in water filtered through some of the same sand, which had however been kept in a jar of water ten days, were increased 5.1% in size. In the next experiment, No. 64, in which the water was filtered through some of this sand which had been heated to 70° so as to kill the vegetable growth in it, the larvæ were slightly increased in size, in spite of the organic ammonia present being nearly quadrupled. This favourable effect was probably in part due to the mechanial removal of suspended organic matter from the water, and in part, as we shall see later, to the removal of bacteria. In the next experiment in the table, No. 65, a fine sand fresh from one of the Aquarium tanks was used, and the larvæ grown in the water filtered through it were increased 5.4%. In Exp. 99, in which a sand which had been stagnating some time in a small tank was used, the larvæ were only very slightly affected. In Exp. 100 an impure sand from the sea shore near the harbour was used, and the ammonia in the water

filtered through it was largely increased. Nevertheless the larvæ were increased 4.7% in size. In Exp. 101 sand dug up a metre below the surface of the sea shore was used. This sand contained no vegetable growth, and must have acted on the water as a mechanical filter pure and simple. The larvæ grown in the water filtered through it were 1.8% larger than the normal, and hence it may reasonably be concluded that when in the other experiments the larvæ were increased in size to a greater extent than this, the additional growth was due to an actual chemical purification to the water, as distinguished from a merely mechanical one. In Expts. 123 and 124 the water was filtered through the apparatus, described above, which contained sand taken from the Aquarium, whilst in Expts. 125 and 126, as also in the above mentioned Exp. 101, it was filtered through a similar apparatus containing sand from a metre below the surface. The samples of water used were collected on the 29th day of the above described experiments. In Exp. 123, in spite of the fact that 86% of the free ammonia was removed, the larvæ were only very slightly increased in size; not so much in fact as in Exp. 124, in which, owing to the slowness of the filtration, the organic ammonia was slightly increased in amount, and the free ammonia more than quadrupled. These apparently contradictory results, as the evidence adduced later on would suggest, may perhaps be ascribed to bacterial action. In Expts. 125 and 126 on the other hand, the larvæ are considerably increased in size. Thus in Exp. 125, in which the water filtered through rather slowly, owing to the formation of a vegetable crust on the top of the sand, the larvæ were increased 8.5% in size. In Exp. 126 the water filtered through much more rapidly, as this top layer of sand, to a depth of half a centimetre, was scraped away. The apparent purifying effect on the water was however even greater than before, but the larvæ were increased by only 4.2%, or less than half as much as before. It should be noted that in all these four last experiments the number of larvæ developing from the ova was much greater than the normal, it being on an average 87%.

As a general result therefore it may be concluded that by filtration of the water through sand impregnated with vegetable growth, it is not only rendered chemically much more pure, but, as far as can be judged by the growth of these Plutei, physiologically purer as well. At the same time it must be admitted that as far as the results given in the above table can show, there is no general and constant relation between the degree of chemical purification

effected, and the ‚physiological' purity. In Expts. 100 and 124, in which the free ammonia in the water was largely increased in amount, the larvæ were nevertheless appreciably increased in size, whilst in Expts. 99 and 123, in which it was very largely reduced, the effect was practically nil. We can only conclude, from this want of uniformity in result, that there is another factor present, which thus far has not been taken into account. This factor, as will presently be made evident, is in all probability of a bacterial nature.

b. The Effect of keeping the Water in Diffuse Daylight.

As has been shown above, a vegetable growth is gradually deposited on sand through which a stream of water is allowed to filter. It follows therefore that the water itself contains small quantities of algæ and diatoms in suspension. In order to determine something further as to the purifying capacities of these minute quantities of vegetable matter upon the water, and as to the rapidity with which they can act, a large covered jar of the ordinary Aquarium water was allowed to stand in a room where it was exposed to no direct sunlight. The chemical analyses made from time to time gave the following results.

	Free NH_3	Organic NH_3
Original water	.223 mgm.	.134 mgm.
After 10 days	.134	.171
- 25 -	.017	.269
- 33 -	.010	.227

These figures show that the free ammonia present in the water is very rapidly removed by some agency or other, only about 1% of it being left after 33 days exposure to diffuse light. The organic ammonia on the other hand is considerably increased, it being double its original amount after 25 days exposure. That these striking variations in the composition of the water are due chiefly, if not entirely, to a vegetable agency, is rendered probable by the fact that after the water had been standing a few days, small patches of vegetable growth, some of them bright green, and others purple brown in colour, began to form on the sides of the vessel, and these patches steadily increased in size as the experiment continued. On microscopical examination, this growth did not appear to be of a definite character, but in the deposit at the bottom of the jar there were a few remains of diatoms. As the jar containing the water had been

very carefully washed out previous to its introduction, this vegetable growth must have arisen from the water itself. The comparatively quick purification of the water must have been due to the rapid increase of the originally very small quantities of vegetable growth present.

In another experiment, of a similar nature to the above, the following results were obtained.

	Free NH_3	Organic NH_3
Original water	.146 mgm.	.114 mgm.
After 13 days	.139	.112
- 24 -	.048	.144
- 36 -	.008	.161
- 50 -	.030	.195

In this case the rates of diminution of the free ammonia on the one hand, and of increase of the organic ammonia on the other, are not nearly so rapid as in the previous instance. This was probably due to the water having contained less vegetable matter, for the growth deposited on the sides of the jar was very much smaller in amount. Microscopical examination showed it to consist entirely of diatoms, and a small amount of structureless greenish brown alga, and not any of the bright green or purple alga noticed in the previous instance. The temperature of the water during the experiment was about 20° to 23° in the former instance. In the present case it was about 25° for the first three weeks, then about 20° for the next fortnight, and about 17° for the remainder of the time. As far as this factor is concerned therefore, one would expect the rate of purification to be more rapid in the second experiment than in the first, the growth of the vegetation being presumably more rapid at a higher temperature.

The results obtained on the growth of larvæ in water which had been exposed to diffuse light agreed with the chemical analyses in proving that a marked purification of the water had taken place. Thus in Exp. 73, the fertilised ova were allowed to develop in some of the water obtained in the first of the above experiments, after 33 days exposure. They proved to be no less than 21.4% larger than those grown in normal Aquarium water! However, the favourable effect was not so pronounced as one might be inclined to judge from this increase in size, as only 35% of the ova used, or about half the normal number, developed into eight days larvæ. In Exp. 129 on the other hand, in which was used some of the water obtained

in the second of the above experiments after 27 days exposure, 71% of the ova developed to larvæ, but these larvæ were only 5.4% larger than the normal. These observations serve to prove therefore, that on exposure of the water to diffuse light for some days or weeks, a very considerable purification, as judged by both chemical and physiological standards, is effected by the rapid growth and multiplication of the small quantities of vegetable matter in suspension in the water.

The Influence of Bacteria on the Purification of Water.

a. The Effect of Sunlight.

In the experiments just described, the water was exposed only to diffuse light. The effect of exposure to direct sunlight was also determined. In two series of observations, a layer of water about 12 cm. in depth was placed in a large covered glass jar, whereby a considerable surface of water was exposed to the air. This jar was exposed the whole day to direct sunlight. Analyses of the water from time to time gave the following results.

	Ammonia per litre in mqm.			Ammonia per litre in mqm.	
	free	organic		free	organic
Original water	.223	.134	Original water	.227	.167
After 2 days	.248	.232	After 5 days	.098	.186
- 4 -	.281	.202	- 12 -	.261	.684
- 10 -	.165	.365			

In the first experiment, given in the left half of the table, the free ammonia at first increased somewhat in amount, and then decreased, whilst the organic ammonia increased rapidly. In the second experiment, the free ammonia at first diminished, and then increased in amount, whilst the organic ammonia rapidly increased as before, it being quadrupled after twelve days exposure. In these two series of experiments, the water was exposed to the action of the air, as well as that of the sun. Two other series were made, in which the water was placed in a flask filled up to the neck, whereby only a very small surface was exposed. After removal of a volume of water

for analysis, the remainder was put into a smaller flask, so that there was still very little exposure to the air. The results of the chemical analyses were however practically the same as before.

	Ammonia per litre in mqm.			Ammonia per litre in mqm.	
	free	organic		free	organic
Original water	.223	.134	Original water	.227	.167
After 4 days	.260	.123	After 5 days	.198	.162
- 10 -	.119	.176	- 12 -	.139	.661

In each case we see that the free ammonia present varies a great deal, though after 10 or 12 days it is considerably diminished in amount, whilst on the other hand the organic ammonia is largely increased. These changes in the composition of the water are due, at any rate in part, to the vegetable growth which begins to form after a few days on the sides of the vessels, in spite of the exposure to the sun, and the high temperature which the water thereby attains. For instance, the temperature of the water in the jar was noticed once or twice to be as high as 38°, and that of the flask, 35°.

Chemical analysis therefore fails to show much difference between the effects of exposure of the water to the sun alone, and to exposure to the air in addition: but on growing larvæ in specimens of water treated in these two ways, the difference is extremely marked.

Number of Exp.	Conditions of exposure of water	% Larvæ formed	% Variation in size of larvæ.	% Varition in amount of	
				free NH_3	organic NH_3
25	Sun + air 4 days	25	— 1.4	—	—
37	- - 4 -	7	+ .6	+ 26	+ 51
61	- - 5 -	7	+ 14.1	— 57	+ 14
62	- - 5 -	4	— 18.0	± 0	— 10
—	- - 12 -	0	—	+ 15	+ 310
26	Sun only 4 days	95	+ 14.6	—	—
38	- - 4 -	83	+ 18.0	+ 25	+ 11
60	- - 5 -	9	+ 17.4	+ 12	— 9

From this table one may see that whilst on an average larvæ grown in water which had been exposed to sun and air are practically unaffected, those grown in water exposed to sun only are increased in size by from 14.6% to 18.0%. The percentage numbers of ova developing to larvæ differ to almost as marked an extent. Thus in the one case on an average only 11%, or, if the experiment in which no larvæ at all were obtained be included, 8.0%, developed, and in the other case 62%. The individual values obtained with water exposed to sun and air vary very considerably. Thus in the first two given in the table the size of the larvæ was practically unaffected; but in the next one it was largely increased, and in the next, still more largely diminished. In this case a layer of water only two inches in depth was exposed in a flat dish. In another experiment in which was used some of the water employed in Exp. 61, but which had been exposed to the sun an additional seven days, no larvæ at all were obtained. It may be thought that the adverse effect of the water thus treated was due to evaporation and consequent increase of salinity. This could not have been the case however, as in the first place the jars in which the water was exposed were always covered up with glass lids, and moreover it has been shown in the paper already referred to above[1] that moderate increase in the salinity of the water has practically no influence on the growth of the larvæ.

But how, on the other hand, can the very favourable effect produced by the exposure of the water to the sun in a flask be accounted for? It is probable, as will be shown later on, that a small part of this influence, probably about 6% of it, is due to the heating of the water by the sun, whereby the animal life present is killed off. There must obviously be some other factor at work however, to which these striking differences in the effects of the water are due. Everything points to this factor being of a bacterial nature. Thus it was originally shown by Downes and Blunt[2] that sunlight had an adverse effect on bacterial growth, and in recent years Marshall Ward has made numerous investigations on the bactericidal action of sunlight[3]. His experiments were made with pathogenic or fresh water bacteria however. Observations on marine

[1] ibid. pag. 588.

[2] Proc. R. Soc. London Vol. 27 1877 pag. 488, also Vol. 25 1878 pag. 197.

[3] Proc. R. Soc. London Vol. 52 pag. 393, also Vol. 53 pag. 23, also Vol. 56 pag. 315.

species were made by Russell[1], and also by B. Fischer[2], who found, as had Buchner for fresh water germs[3], that diffuse daylight had a certain amount of germicidal power, as well as direct sunlight. Fischer indeed showed that the numbers of bacteria in specimens of surface sea-water collected in the evening, after the whole day's exposure to the sunlight, were considerably smaller than in those taken in the morning. In the present research the numbers of bacteria, in various specimens of water, variously treated, were determined by the ordinary gelatin plate culture method. As the observations were begun in the summer, when the temperature of the moist chamber remained at about 25°—26°, it was thought better, as the weather became colder in the autumn, to continue to keep the cultures at this same temperature. Hence the temperature of the chamber was artificially kept up to this point by means of a gas flame and regulator. The bacteriological examination consisted merely in determining the numbers of colonies formed after 24 and 48 hours respectively, and no attempt was made to isolate and study the various bacterial forms.

With regard to the effects of sunlight on water, the bactericidal action noticed by previous observers was confirmed. Thus on exposing a flask full and a covered jar half full of the Aquarium water to sunlight, for some days, the following numbers of bacteria per cubic centimetre were found.

Time of exposure	Water in jar		Water in flask	
	24 hours	48 hours	24 hours	48 hours
Original water	1500	3340	1500	3340
After 4 days	270	420	450	4730
- 10 -	840,000	—	900,000	—

Here we see that after four days exposure, the number of bacteria was considerably diminished in one case, but in the other only the rapidity of growth of the colonies was affected, the actual number of colonies after 48 hours development being increased. After ten days exposure to the sun however, the numbers of bacteria present were in each case enormously increased. The sun was not very

[1] The Botanical Gazette Vol. 18 1893 pag. 414.

[2] Die Bakterien des Meeres. in: Ergebn. Plankton-Exped. Bd. 4 M 1894 pag. 55.

[3] Arch. Hyg. 17. Bd. pag. 197.

powerful during the latter part of the experiment, and towards the end the sides of the vessels became coated with a thin layer of brown algæ. In the next experiment the following results were obtained.

Time of exposure	Water in jar		Water in flask	
	24 hours	48 hours	24 hours	48 hours
Original water	1180	2200	1180	2200
After 3 hours	440	3800	270	1200
- 6 -	—	—	250	940
- $3\frac{1}{2}$ days	240	2400	130	2000
- 15 days	—	—	10,500	22,000

Here again the rate of growth of the bacterial colonies was considerably slowed down by even three hours exposure to the sun, but after 48 hours, they were on an average as numerous as in the original water. The countings made after 6 hours and $3\frac{1}{2}$ days show distinct germicidal action. Nevertheless, after 15 days exposure, the number of bacteria had increased again as it did before, and similarly also the sides of the vessel were coated with brown alga. In both these series of experiments, the vessels of water were exposed directly to the sun, and the water in them thereby became heated up to sometimes as high as 38° C. At these temperatures the rate of multiplication of the surviving bacteria would of course be much greater than at normal temperatures, and hence actually occurring germicidal action would be partially or entirely masked. In the next two series of experiments therefore the jar and flask of water were placed in a larger glass vessel, through which circulated a continuous stream of fresh water. By this means the temperature was generally kept at about 18°, and was never noticed to be higher than 21°. The results were as follow:

Time of exposure	Water in jar		Water in flask	
	24 hours	48 hours	24 hours	48 hours
Original water	1130	2300	1130	2300
After 2 hours	270	—	130	—
- 6 -	40	—	50	—
- $5\frac{1}{2}$ days	90	870	450	850

In this experiment some of the countings after 48 hours incubation were by an accident omitted. Those made after 24 hours show a very rapid diminution in the number of germs. Thus on an average about five sixths of them were killed off or rendered slowly growing by a two hours exposure to the sun, and only 4% survived six hours' exposure. After five and a half days of almost continuous sunlight however, the numbers had increased again, so that on 48 hours' incubation the colonies obtained averaged more than a third of the original number. The next experiment, made under the same conditions, gave the following results:

Time of exposure	Water in jar		Water in flask	
	24 hours	48 hours	24 hours	48 hours
Original water	1740 / 1560	3500 / 2700	1650	3100
After 1 hour	1560	2260	1070	2520
- 3 hours	440	1360	310	1700
- 6½ hours	350	1450	330	2100
- 3½ days	270	5500	35	13,600
- 7½ -	—	150	—	310
- 10½ -	—	70	—	180
After 12½ days	250,000	320,000	10,000	12,400
- 14½ -	170	950	2600	5100

These figures show that an appreciable number of bacteria were killed by even one hour's exposure to sunlight, whilst after three hours the number of colonies obtained on 24 hours' incubation averaged less than a quarter of the original number, and after 48 hours' incubation, about a half. A further 3½ hours' exposure seems to have had no further action, whilst after 3½ days, the number was very much larger than that originally present. This increase was probably owing to the sunlight during the intervening days having been only very occasional. For the next several days it was continuously bright, and hence we see that after four more days' exposure the number of bacteria had diminished, on an average, to about 7% of the original number. After a further three days' exposure, the number of germs was still further diminished. The vessel containing the jar and flask of water was now taken away from the direct sunlight, and kept in

a badly lighted room. Here the temperature remained fairly constant at about 15° C. Nevertheless, after keeping the water two days, the number of germs was found to have increased enormously in both specimens of water, but especially in that kept in the jar. After a further two days, the numbers had diminished again, though not to so great an extent as they had previously increased. It would seem therefore that immediately the direct rays of the sun are removed from the water, the germs still unharmed begin to multiply with extreme rapidity, so that the water soon becomes bacterially much more impure than before. These rapidly multiplying germs then rapidly die off, so it is possible that the ultimate bacterial quality of the water may be somewhat better than originally; but even this is doubtful. As the number of germs begins to increase so rapidly after the germicidal sunlight is shut off from the water, the cultures were generally made late in the afternoons, after the water had been exposed the whole day to the sun. A determination of the bacteria present after $3^1/_2$ or $5^1/_2$ days' exposure would thus give very much smaller numbers of germs than if it had been made after 4 or 6 days, for then the water would be examined after it had been exposed for only an hour or so to the early morning sun.

As to the primary object with which these observations were made, no information is afforded. Thus on comparing the various numbers obtained for water exposed in a jar to the air, with those for water exposed in a flask, no constant or appreciable difference in either direction is found. These countings of course show nothing as to the quality of the bacteria in either case, hence one may be allowed to explain the great differences effected on larval growth by the water treated in these two ways as due to the different degrees of oxygen tension in the water. Thus these may have caused an increase or decrease in the number of certain germs with which the vital processes of the larvæ are closely bound up.

b. The Effect of Keeping the Water in Darkness.

Inasmuch as by far the larger portion of the ocean water is found at depths whence no trace of light can penetrate, it was thought to be of interest to determine what changes, if any, would take place in sea-water kept in darkness. Accordingly, a large covered jar of Aquarium tank water was placed in a cupboard, and chemical analyses of it made from time to time. It should be stated that this

water was not in absolute darkness, as there were one or two cracks in the woodwork, through which minute quantities of light could enter. The analyses were as follow:

	Free NH_3	Organic NH_3
Original water	.227 mgm.	.167 mgm.
After 10 days	.205	.195
- 22 -	.039	.098
- 61 -	.020	.085
- 85 -	.005	.092

Here we see that, by the simple process of keeping the water in darkness, no less than 98% of the free ammonia and 45 to 49 of the organic ammonia, were removed! In another jar of the same water, placed at the same time under conditions of absolute darkness, the change in composition was as follows:

	Free NH_3	Organic NH_3
After 10 days	.201 mgm.	.140 mgm.
- 22 -	.027	.100

In this case the removal of the ammonia seems to have taken place rather more rapidly.

In the next experiment, the jars of water were wrapped up in black paper, and placed in a cupboard, so that by no possibility could a trace of light reach them. One jar contained ordinary Aquarium water, another contained water which had been filtered through asbestos (in order to determine if there were any diminution in the rate of purification owing to the removal of bacteria), and the third also contained Aquarium water, but to it had been added about 50 cc. of a water which had previously been kept 31 days in darkness, for it was thought possible that this water might contain numbers of bacteria possessing a special capacity for removing the ammonia from water. The analyses gave the following results.

	Aquarium water		Filtered water		‚Seeded' water	
	Free NH_3	Organic NH_3	Free NH_3	Organic NH_3	Free NH_3	Organic NH_3
Original water	.340	.135	.395	.225	.340	.135
After 25 days	.007	.065	.008	.069	.005	.059
- 54 -	.008	.068	.009	.069	.007	.065

From these figures we see that after 25 days an apparently limiting value for the purification of both the organic and the free

ammonia has been reached, for after an additional 29 days no further purification resulted. The degree of purification seems to have been practically the same for all three waters.

This very marked purification of water kept in darkness is, we must conclude, almost entirely due to bacterial action. Thus some of the same Aquarium water, kept under the same conditions as the above, but to which .2% of corrosive sublimate had been added, was found on analysis after 26 days to contain .255 mgm. of free, and .120 mgm. of organic ammonia, or respectively 25% and 11% less than the original water. This diminution was probably due to the vaporisation of some of the ammonia from the water, and to coagulation and partial fixation of the proteids in the organisms present in the water, which on distillation would otherwise have evolved some ammonia.

Still two other jars of water were kept in absolute darkness under conditions similar to the above. One of these jars contained Aquarium water which had been heated to boiling point in a corked flask, and then cooled rapidly, so that practically none of the ammonia contained in it escaped. The analyses were as follow:

	Free NH_3	Organic NH_3
Original water, after heating	.350 mgm.	.202 mgm.
After 26 days	.218	.125
- 54 -	.020	.090

Here we see that after a period of 26 days, which was sufficient in the other cases for the maximum purification of the water to be effected, only a small proportion of the ammonia was removed, and that even after 54 days the limit of purification was not reached. This delay in the purification was obviously due to the bacteria in the water having been killed off by the heat. The water had been poured into an unsterilised jar, recently washed out with sea-water, hence there were doubtless sufficient bacteria present which could, on subsequent multiplication, exert their purifying action.

The other jar kept in darkness was also filled with Aquarium water, but the jar itself contained a layer of green and purple brown algæ gradually deposited on it from Aquarium water after it had stood for 36 days in diffuse light. After 26 days in darkness, this layer of algæ had diminished in amount, whilst the water itself was found to contain .007 mgm. of free, and .126 mgm. of organic ammonia, or respectively 98% and 7% less than the original water. As we have already seen, the organic ammonia in water kept in diffuse

light is gradually increased by the action of the algæ. In the present instance there would presumably be this action at work tending to increase the organic ammonia present, and a bacterial one tending to decrease it. The net result of these two opposing actions has been to leave the organic ammonia almost unchanged.

It was remarked above that in the parallel observations made with one jar kept in semi-darkness, and the other in absolute darkness, the rate of purification seemed to be rather more rapid in the latter case. That this is actually the case is confirmed by the following two series of observations, in both of which the jars of water were first kept for 28 days in almost absolute darkness, and then for the rest of the period kept in a cupboard, which had several cracks in it, through which a fair amount of daylight filtered. In one of these experiments ordinary Aquarium water was used, and in the other, some of the same water filtered through asbestos.

Treatment of water.	Aquarium water		Filtered water	
	Free NH_3	Organic NH_3	Free NH_3	Organic NH_3
Original water	.146	.114	.115	.172
After 14 days in darkness	.099	.100	.119	.106
- 25 - - -	.010	.097	.005	.110
- 37 - - semi-darkness	.007	.131	.005	.120
- 52 - - -	.040	.124	.008	.133
- 67 - - -	—	—	.005	.112

Here we see that in both instances the organic ammonia in the water begins to increase slightly in amount after the transference of the water from darkness to semi-darkness. This does not seem to have been due to the formation of vegetable growth in the jars. At least there was no visible trace of it after three weeks exposure to semi-darkness. It may be concluded therefore, with a fair degree of probability, that the bacteria exert their purifying action on the organic ammonia, and possibly also upon the free ammonia, most efficiently in absolute darkness.

In connection with these experiments, the conclusions of other observers on the purifying effects of fresh water bacteria may be recorded. It should be noted however, that most of these and other observations not here mentioned[1] differed from the above experiments

[1] Tiemann und Gartner, Untersuchung des Wassers 3. Aufl. pag. 497.

with sea-water, in that they were made in the presence of light. Thus Uffelmann has shown[1] that these bacteria may oxidise the ammonia in the water, more or less irrespective of the presence of air. Heraeus has shown[2] that some kinds of bacteria exert an oxidising action, and others a reducing one. Again, S. Leone, who worked with distilled water containing a little gelatin, found that the organic substances were gradually decomposed into inorganic compounds, first ammonia, then nitrous acid, and then nitric acid, being in turn formed[3]. This subject of nitrification, with reference to sea-water, will be discussed in this paper further on.

It was remarked, whilst discussing the second series of the above experiments, that the purification of the water appeared to reach its limiting value after 25 days. The three specimens of water (Aquarium water, filtered water and ‚seeded' water), then contained respectively .007, .008 and .005 mgm. of free ammonia per litre, and .065, .069 and .059 mgm. of organic ammonia. It was thought to be of interest to determine what relation pure open sea-waters bore in their composition to these bacterially purified waters, so the following analyses were made.

Distance from shore, at which water was collected	Date	Free NH_3	Organic NH_3
1 kilometre	June 11th	.054	.105
2 kilometres	-	.015	.094
3 -	-	.010	.062
5 -	Oct. 8th	.008	.072
15 -	Nov. 5th	.004	.071

These analyses show that open sea-water practically reaches its limiting degree of purity at about 3 kilometres from the shore, and also, that this degree of purity is practically the same as that which was in some cases effected in Aquarium tank water by bacterial action in the darkness. This is a significant fact, as it seems to show that by merely keeping impure water for a few weeks in darkness, as great a degree of purity may be reached as is found in the open sea, where the water is

[1] Arch. Hyg. 4. Bd. H. 1.

[2] Zeit. Hyg. 1. Bd. pag. 193.

[3] Gazetta Chimica Italiana. 1887.

exposed to every favourable purifying influence. It would be of great interest to analyse a specimen of water collected at a considerable depth beneath the surface, to see if in this case also the degree of purity was no greater. Unfortunately however I was not able to obtain one.

Another proof that the limit of purity of the ammonia in the water is that which is sometimes obtained by keeping water in darkness, is afforded by the analyses of a specimen of open sea-water t ken 5 km. from the shore. This was kept in absolute darkness, in a jar covered with black paper.

	Free NH_3	Organic NH_3
Original water	.008	.072
After 20 days	.021	.082
- 28 -	.005	.062
- 40 -	.040	.076

From these figures we see that even after 40 days in darkness, practically no purification was effected. In fact, the water contained more ammonia than at first. There was obviously some bacterial action taking place in the water, however, as the variations in the amount of free ammonia present are much too great to be referred merely to experimental error.

The purification effected by keeping water in darkness, as tested by its influence on the growth of sea-urchin larvæ, is very striking. The results obtained are collected in the following table.

Number of Expt.	Conditions under which water had been kept	Amount, in purified water of		Amount, in normal Aquarium water of		% of larvæ formed	% variation in size of larvæ
		free NH_3	organic NH_3	free NH_3	organic NH_3		
73	23 days in semi-darkness	.039	.098	.350	.125	45	+ 17.8
79	23 days in darkness	.027	.100	-	-	88	+ 17.4
82	25 - - -	.007	.065	.193	.143	45	+ 4.2
83	25 - - - (‚seeded' water). . . .	.005	.059	-	-	70	+ 11.4
84	25 - - - (in jar containing algæ)	.007	.126	-	-	15	+ 5.0
86	25 - - - (after heating to 100°C.)	.218	.125	-	-	37	— 3.4
118	55 - - - - - - - - -	.020	.090	.251	.129	38	+ .1
116	55 - - -	.008	.068	-	-	54	+ 3.0
119	55 - - - (‚seeded' water)	.007	.065	-	-	27	+ 5.6
128	27 - - -	.010	.097	.074	.094	72	+ .4

The values given for the ammonia in the normal Aquarium water are for the specimens of water in which were grown the larvæ taken as ‚normal', and against which the larvæ grown in the waters exposed to darkness were compared. They do not represent the composition of the water before purification by keeping in darkness.

From the values given in the last column of this table, one may see that in almost all cases the larvæ were considerably increased in size, in two instances by more than 17%, but as a rule by about 5%. The great increase in size in the first two experiments given in the table was probably in part due to the dwarfing of the ‚normal larvæ, by the very impure water in which they were grown. Thus in Exp. 82, in which the water had been purified to a much greater extent, the larvæ were only 4.2% larger than the normal, but on the other hand the water in which the normal larvæ were grown was much purer than before. In Exp. 83, the water used had been kept in darkness for the same length of time as that in Exp. 82, but, as already mentioned, it was previously ‚seeded' by the addition of a small quantity of water which had itself been kept in darkness for 31 days. With this water the larvæ were increased by 11.4%, so that the seeding seems to have induced an increased physiological purification. In Exp. 86 was used the water which had, before keeping in darkness, been heated to 100° C. The larvæ are in this instance actually diminished in size by 3.1%, whilst in a sample of the same water after 55 days darkness, and in which very much more of the ammonia present had been absorbed, the larvæ were practically unaffected. In Expts. 116 and 119 were employed samples of the same water as was used in Expts. 82 and 83, which had however been kept an additional 30 days in darkness. The favourable effects produced were not so great as before, the larvæ being increased in size by respectively 3.0% and 5.6%, instead of 4.2% and 11.4%. This may have been due to the water in which in the ‚normal' larvæ were grown being more favourable to larval growth in the latter instance than in the former. In any case one is justified in concluding that the water had undergone little or no increase in ‚physiological' purity during the extra 30 days, just as it had undergone no increase of chemical purity.

Lastly in Exp. 128 the larvæ were only very sligtly affected, but it should be noted that the Aquarium water was on this occasion even purer, in regard to its organic ammonia, than the water kept in darkness.

In the last column but one of the table are given the percentages of fertilised ova which reached full larval development. If Expts. 84, 86 and 118 be excluded, it appears that only 53% of the ova developed to the pluteus stage, or somewhat less than the normal number (66.6%). The favourable effect produced by keeping water in darkness is therefore not quite so great as one might conclude from the increased size of the larvæ.

It has been shown that water kept in darkness may sometimes become as pure, so far as its ammonia is concerned, as open sea-water taken several kilometres from shore. Judged by the physiological test however, its purity is not nearly so great. Thus in Exp. 69, in which water collected 3 km. from shore was used, the larvæ were 19.2% larger than the normal, and in Exp. 134, with water 5 km. from shore, 12.7% larger than the normal. The increase in size is therefore considerably greater than with waters kept in darkness. The number of ova reaching full larval development was also much larger, it being respectively 100% and 95% in these two experiments.

In addition to a chemical and physiological purification, the keeping of water in darkness leads to a considerable diminution of the bacteria present. In the following table are given the numbers of colonies, per cubic centimetre of water, formed after 24 and 48 hours incubation.

Days water was kept	Conditions	Nature of water	Number of colonies after	
			24 hours	48 hours
30	darkness	normal	liquefied	—
39	-	-	1070	3930
54	-	-	260	1560
30	-	filtered through asbestos	770	1320
39	-	- - -	800	1760
54	-	- - -	140	270
30	-	seeded water	78	780
38	-	- -	210	730
30	-	from jar containing algæ	72	390
30	-	heated to 100°	1340	6000
39	-	- - -	580	620
54	-	- - -	270	1210
74	semi-darkness	normal	55	1970
145	-	-	18	130
24	darkness	-	360	3900
28	-	-	600	1500
24	-	filtered through asbestos	18	840
65	-	- - -	270	650

As will presently be shown, the number of bacterial colonies formed from ordinary Aquarium water after 24 hours' incubation is about 1500, and after 48 hours', about 3000. From this table we see that with waters kept in darkness, the numbers are scarcely ever as high as these, they being generally only a few hundred after 24 hours' incubation, and in five cases out of eighteen, less than a hundred. After 48 hours however, the number is more often than not over 1000, and hence it would seem that the rapidity of multiplication of the bacteria on gelatin plate culture has been very markedly diminished in addition to the actual number. With regard to individual experiments, the first two thirds of the table consists of observations made with jars of water kept in darkness under similar conditions, but containing respectively ordinary Aquarium water, water filtered through asbestos, water seeded with other water previously kept in darkness, water placed in a jar covered with a deposit of algæ, and water previously heated to 100° C. Of these five samples, the seeded water and water in the jar coated with algæ contained the least number of bacteria, and the water heated to 100° the most. The comparatively large number of germs present in this latter water is of interest in connection with the fact that the larvæ grown in it were, in contradistinction to those grown in the other specimens of water, considerably smaller than the normal. Determinations made 9 days after these original cultures showed the numbers of bacteria to be about the same. Those made 24 days later, on the other hand, showed a distinct decrease. In the observation given in this table on a specimen of water kept 74 days in darkness, the number of colonies formed after 24 hours is smaller than usual, but after 48 hours is just as large. After a further 71 days however, the diminution in number is very conspicuous. Again, in a specimen of water filtered through asbestos, the number of colonies obtained after keeping 65 days is somewhat smaller than after 24 days.

It may therefore be concluded that the longer a water is kept in darkness, the smaller becomes the number of bacteria it contains, and the slower is their rate of multiplication on plate cultivation.

On the bacteriology of open sea-water no observations were made, as the subject has already been worked out by Russell[1] for waters in the Bay of Naples, and very fully by Fischer for Atlantic Ocean waters[2]. Russell found the number of bacteria in surface

[1] Zeit. Hyg. 11. Bd. 1891 pag. 165.

[2] ibid. pag. 1—83.

water taken at from 4 km. to 15 km. from the shore of Naples to vary between 6 and 78 per c.c., but the numbers obtained seemed to have little or no relation to the distance from the shore. Also the number of bacteria seemed to vary but little with the depth of the water, it being practically the same for water at a depth of 825 metres as for surface waters. Again, Fischer found the number of germs at 200 and 400 metres depth to be as a rule considerably greater than the number in surface water. At greater depths the numbers rapidly diminished, so that below 1100 metres only 1 germ per cubic centimetre was as a rule present. Fischer concluded, however, that whatever the depth, the water was never absolutely germ-free. This occurrence of bacteria in water at all depths is of interest with reference to the results obtained with water kept in darkness. Thus it is obvious that as they are always present, they will inevitably exert their purifying action on any nitrogenous contamination communicated to the water, and so keep the water at its highest possible limit of purity.

It is of interest to inquire how far this favourable influence of keeping water in darkness enters into the purification of the water of Marine Aquaria. At the Naples Aquarium the water runs back from the tanks into one of three reservoirs, which hold in aggregate about 300 cubic metres. Here it remains from one to three days, when it is again pumped into the various tanks. On making analyses of this water pumped from a reservoir, and of that flowing back again from the various tanks, it was always found that the latter was much more impure than the former. After making numerous analyses of water when it had just run into the reservoirs, and after it had stood in them for a day or two, it was not however possible to establish any constant difference of composition. This, if it were present, would in any case be slight, as never more than two thirds of the water is pumped away from a reservoir before the water from the tanks is allowed to run back again. How then was the observed purification of the water brought about? It was eventually found that this removal of ammonia from the water was effected during its passage along the lead pipes running from the subsidiary reservoirs at the top of the buildings into the various rooms. Thus the tap from which the water used in these experiments was drawn was about 20 metres distant from the supplying tank. In one instance the reservoir water contained .162 mgm. free, and .185 mgm. organic ammonia, and the water drawn at the same time from the tap, at

the rate of a litre in 10 minutes, only .075 mgm. free, and .125 mgm. organic ammonia, or respectively 54% and 32% less. In another instance the water was drawn from the tap at varying rates, in order to determine the effect of the rapidity of flow on the amount of ammonia removed. The following results were obtained.

Nature of water	Free NH_3	Organic NH_3	% Difference in free NH_3	% Difference in organic NH_3
Water of reservoir	.188	.131		
Drawn from tap: 1 l. in 10″	.140	.098	— 26	— 25
- - - 1 l. in 2^h 40′	.034	.110	— 82	— 16
- - - 1 l. in 3^h 30′	.057	.107	— 70	— 18

Here we see that when the water was allowed to run at its maximum rate from the tap, 26% of the free, and 25% of the organic ammonia was removed. When however the water was allowed to drip slowly from the tap, no less than 82% of the free ammonia was in one case removed. The rate of flow in these cases was really much faster than that given, as from the same pipe another stream of water was running at the rate of a litre in 36 seconds. The exit tube of this jet was situated about 16 metres from the reservoir, hence the water analysed only flowed at the very slow rate given above through a length of four metres of pipe. The total internal area of the whole pipe was calculated to be roughly about one and a third square metres, and hence it follows that exposure of a litre of water to this area of the bacterial slime which coats the inside of the pipes, was able, in ten seconds, to remove a quarter of the free and organic ammonia present. This seems an extremely rapid rate of purification, yet it should be remembered that in the previously described experiments with sand kept in darkness, it was found that water could be filtered at the rate of a litre in ten minutes, and yet have 87% of the free, and 20% of the organic ammonia removed.

In still another determination, water running from the tap at the rate of a litre in 47 minutes, was found to contain 30% less free, and 3% less organic ammonia than the water flowing at a litre in 12 seconds.

As the bacterial slime which covers the pipes is so effectual a purifying agent, it was thought that the brown vegetable matter which is deposited from the water on standing in the reservoirs

might possibly have a similar effect. To test this point, a small quantity of the deposit, amounting in all to only one or two grams, was dredged up from one of the reservoirs, and was stirred from time to time with about 700 cc. of water, for half an hour. After standing a few minutes, the water was filtered off and analysed. It was found to contain .657 mgm. of free, and 1.490 mgm. of organic ammonia, in place of the respective amounts of .286 mgm. and .157 mgm. originally present. So far from this deposit being a purifying agent, it is therefore a most fertile source of contamination. Thus the deposit had been collecting for only two months, previous to which the reservoir had been thoroughly cleaned out. Probably if it had been standing a longer time, its fouling properties would have been much greater.

On comparing the effect on larval growth of water drawn from one of the pipes with that taken directly from the large tank of the Aquarium, the increase in physiological purity proved to be no less marked than the increase in chemical. The following were the results obtained.

Number of Experiment	% Variation of NH_3, on that present in normal tank water		% Larvæ formed	% Variation in size of larvæ.
	Free NH_3	Organic NH_3		
75	— 37	— 4	70	+ 10.8
80	— 46	— 21	67	+ 8.2
115	— 42	+ 8	69	+ 4.2

In these three experiments the larvæ were on an average increased 7.8% in size, but the percentage of ova reaching the larval stage was only very slightly increased. In each case the water contained about 40% less free ammonia, but the organic ammonia was but little altered.

The tap water is also bacterially purer than the ordinary tank water. Thus in one case, after 24 hours' incubation, the tap water was found to give 930 colonies, as against the 2000 of the normal tank water. In another case, after 24 and 48 hours' incubation, the tap water gave respectively 580 and 700 colonies, and the normal tank water 1500 and 3340. The number of bacteria seemed also to depend upon the rate at which the water flowed from the tap; but as the results obtained were rather contradictory, it is deemed unnecessary to mention them in detail.

c. The Effect of Filtration through Asbestos.

In some of the observations to be subsequently described, it was necessary to filter off the larvæ from the water in which they were growing. This was done by plugging up the bottom of a funnel with a layer of asbestos about a centimetre in thickness, and letting the water run through. It was accordingly thought advisable to determine whether the filtration itself could have any effect on either the chemical composition of the water, or on the growth of the larvæ. The following results were obtained.

Number of Experiment	% Variation in		% Larvæ formed	% Variation in size of larvæ
	free NH_3	organic NH_3		
77	—	—	66	+ 22.8
98	— 21	+ 51	64	+ 9.0
121	+ 7	+ 16	45	+ 6.0
85	+ 16	+ 56	48	+ 7.4

From these figures one may see that whilst the free ammonia present in the water was only slightly affected, the organic ammonia was considerably increased in amount. This could scarcely have been due to any soluble impurity in the asbestos itself, for half a litre or more of water was allowed to filter through before a specimen was collected. It can only be concluded that the process of filtration itself causes the increase. It may be that the particles of solid organic matter in the water become caught by the asbestos fibres, and broken up and decomposed by the stream of water running through them.

Strange as is the effect on the chemical composition of the water, that on the growth of the larvæ is much more remarkable. Thus in one instance they were increased in size by no less than 22.8%! On the other hand upon the actual number of fertilised ova reaching full larval development, the filtration has either no effect at all, or a slightly adverse one. In the last experiment given in the table, the larvæ were grown in a specimen of water which had been filtered through asbestos, and then kept 25 days in darkness. The increase in size is 7.4%, whilst with larvæ grown in some of the same water, which had also been kept in darkness under the same conditions, but which had not been filtered through asbestos, the increase was only 4.2%, or 3.2% less.

It is difficult to say to what this very marked increase in the physiological purity of the water was due. Thus we have previously seen that filtration of the water through pure sand which contained no vegetable growth, caused an increase of only 1.8% in the size of the larvæ grown in it. Such filtration must have removed all the minute forms of animal and higher vegetable life present in the water, and also a certain proportion of the bacteria, and should, one would think, have acted more efficiently than filtration through a thin layer of asbestos. Thus determinations by means of plate cultures seemed to show that the asbestos filter removed practically no germs from the water. In one case the water before filtration was found to contain respectively 800 and 850 colonies after 24 and 48 hours incubation, whilst after filtration the numbers were 1000 and 1820. In another case the numbers were respectively 380 and 6870 for 24 and 48 hours incubation before filtration, and 700 and 12700 after it. However in the two instances given a few pages back, in which, after filtration through asbestos, the water was kept for 24 to 54 days in darkness, the number of bacteria present is considerably smaller than in the unfiltered water kept under similar conditions. It is probable that the filtration removes a large number of bacteria from the water, but in that particles of organic matter, swarming with bacteria, get caught in the interstices of the asbestos fibres and broken up by the rapid stream of water flowing past them, the apparent number in the filtered water is just as great, or is increased. Thus in a gelatin plate culture, a particle of organic matter containing hundreds of germs would give rise to only one colony, just like a single isolated germ.

d. The Effect of Heating the Water.

Inasmuch as exposure of the water to sunlight, and filtration through asbestos, whereby in all probability many of the bacteria are removed, were found to have such a favourable influence on the growth of the larvæ, it was determined to try the effect of killing off the germs by previously heating the water. By this procedure the condition of the water is altered in other respects besides, as all the animal and vegetable life in the water are killed off, in addition to most of the bacteria. Also a large proportion of the gases dissolved in the water is driven off. However, as has been shown in the

paper already referred to[1], considerable variations in the amounts of free oxygen and carbonic acid dissolved in the water have but little influence on the growth of the larvæ.

The results obtained are given in the following table. In all cases the water was heated in a large flask filled up to the neck, and on the temperature reaching the desired point, this flask was placed under a stream of cold water, so as to cool its contents as rapidly as possible. The water was then poured into a jar, and allowed to stand for 24 hours before the introduction of the fertilised ova.

Number of Experiment	Temperature to which water was heated	% Larvæ formed	% Variation in size of larvæ.	
15	100° C.	4	+ 1.4	6.9
57	100°	85	+ 13.6	
102	100°	73	+ 5.8	
58	77°	66	+ .03	4.0
103	76°	81	+ 8.0	
59	50°	100	+ 8.4	7.1
104	50°	66	+ 5.9	

From these figures we see that in five out of the seven experiments made, there was a very considerable increase in the size of the larvæ, this increase ranging from 5.8 to 13.6%. In every case but one also the percentage of fertilised ova reaching the larval stage was equal to or greater than the normal. It will be seen that two or three experiments were made at each of the three temperatures 100°, 76° or 77°, and 50°, and probably the effect produced is on an average about the same for each temperature. In the experiment made at 77° the larvæ were apparently unaffected, but this result was probably due to some unknown source of error. Also in the first experiment given in the table the larvæ were only slightly increased in size, and the number of ova reaching the larval stage was very small. This was probably due to the water used being not only heated to 100°, but actually boiled for two or three minutes, whereby much more of the dissolved gases was driven off. The heating of the water to 100° probably exerts two opposing influences on the quality of the water, which more or less neutralise each other.

[1] ibid. pag. 597.

Thus it drives off a good deal of the dissolved gases, but also removes some of the ammonia present. For instance, the water used in Exp. 102 contained 36% less free, and 7% less organic ammonia, than the normal water.

These results lead one to conclude either that a temperature of 50° is almost as fatal to those bacteria in the water which exert an injurious action on larval growth as a temperature of 100°, or that the favourable effect produced by heating the water is due rather to the removal of the small amount of animal and higher vegetable life present, to which, rather than to the bacteria, the harmful effects are accountable. As to the actual germicidal effects of heating the water, the observations of MIQUEL[1], made of course with fresh water bacteria, may be cited. Thus in one case, after heating to 50° for about half an hour, there were killed some 84% of the germs: after heating to 80°, 97%, and to 100°, 99.4%.

Bacteriological examination of the water used in Expts. 102, 103 and 104, gave the following results.

Nature of water	Before introduction of ova		After 2 days larval development	After 8 days larval development	
	24 hours	48 hours	24 hours	24 hours	48 hours
Normal tank water	800	850	16,000	1040	1470
Water heated to 100°	2960	8000	12,700	1650	8200
- - - 76°	100	320	27,000	1640	6650
- - - 50°	340	480	7,000	3710	liquefied

From this table we see that, previous to the introduction of the fertilised ova, the water heated to 100° already contained more bacteria than the normal, whilst the specimens heated to 76° and 50° contained considerably less. This unexpected result must have been due to the rapid multiplication of the bacteria in the water during the 24 hours previous to the introduction of the ova. Thus the jars into which the water was poured after heating were unsterilised, and had recently been washed out with sea-water, whence the rapidly multiplying bacteria may have arisen. Doubtless also some of them came from the spores and bacteria unharmed by the heating. After the fertilised ova had been developing two days, the water heated to 76° was found to contain considerably more bacteria

[1] La Semaine Médicale, 31 Juillet, 1884.

than the normal, and the other two specimens of water somewhat less; but when the larvæ had reached their full eight days' development, all three specimens of water were found to contain more bacteria than the normal. Thus the bacteriological condition of the heated water seems to have been little if at all better than that of the unheated. This counting of colonies is, however, unable to distinguish harmful from harmless bacteria, and it is very likely that the heating killed off the most injurious germs, allowing only the harmless ones to subsequently multiply.

e. Other Bacteriological Results obtained.

The present seems a favourable opportunity to mention the results of some of the other bacteriological examinations undertaken. Plate cultures of the normal tank water of the Aquarium gave the following numbers of bacteria.

Date	Temperature of water	Colonies after		Date	Temperature of water	Colonies after	
		24 hours	48 hours			24 hours	48 hours
16. VIII	23.0°	510	liquefied	15. X	17.2°	1180	2200
23. VIII	23.0	3330	liquefied	25. X	17.5	1130	2300
30. VIII	23.4	800	850	1. XI	15.0	1740	3500
10. IX	22.6	2000	liquefied	8. XI	14.3	—	1700
4. X	20.1	1500	3340	10. XI	14.1	—	1270
12. X	16.5	3340	7160				

Here we see that after 24 hours' incubation, the number of colonies varied from 510 to 3340 per cubic centimetre, and after 48 hours', from 850 to 7160. These numbers are somewhat smaller than one would expect. Thus SANFELICE[1] found as a rule very much larger numbers of bacteria in various specimens of water collected close to the shore of Naples, near the openings of drains, and in the harbours. Out of 96 determinations, the water contained less than 1000 bacteria in 27 cases, and more than 100,000 in 15 cases.

Of the other bacteriological examinations, the majority were made to determine the effects of filtration through sand more or less impregnated with diatoms and algæ. The following results were obtained.

[1] Boll. Soc. Natural. Napoli Vol. 3 1889 pag. 33.

Nature of water	Time of filtration of 1 litre	Before addition of ova		After 2 days' larval growth	After 8 days' larval growth	
		24 hours	48 hours		24 hours	48 hours
Normal unfiltered tank water . . .		800	850	16000	1040	1470
Filtered through 16 cm. surface shore sand	1′ 45″	440	740	—	—	—
Filtered through 16 cm. sand from 1 metre beneath surface.	3′ 30″	330	550	1930	1160	7100
Filtered through 16 cm. Aquarium tank sand	3′ 15″	160	840	2180	1750	2040

Here we see that filtration of the water through a layer of sand 16 cm. in depth in each case diminished the bacteria present, but not very markedly. Of the three specimens of sand used, that dug up a metre below the surface of the shore contained no appreciable vegetable growth, and so must have acted merely as a mechanical filter. The other two specimens on the other hand contained small quantities of vegetable matter. After two days' growth of the larvæ, the specimens of filtered water were found to be bacterially very much purer than the normal water, though in every case the number of bacteria had largely increased. After eight days' growth the number had diminished again, but much more for the normal water than for the samples of filtered water, so that these latter now contained more bacteria than the former.

After a current of water had been allowed to run continuously through two of the specimens of sand for 29 days, fresh bacteriological determinations were made.

Nature of water	Time of filtration of 1 litre	Before addition of ova		After 1 days' larval growth		After 8 days' larval growth	
		24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Normal tank water		380	6870	2450	23000	650	1670
Filtered through Aquarium tank sand	1′ 0″	20000	80000	144000	309000	—	2420
Filtered through sand 1 metre beneath surface	8′ 15″	liquefied	—	7000	25000	—	2800

We see that now, after the sand has become impregnated with vegetable growth, the filtration enormously increases the number of bacteria in the water, instead of diminishing it. This increase was

still maintained after the larvæ had grown four days in the water, but after eight days the difference was much less marked. The water filtered through Aquarium sand contained, at any rate after four days' growth, a very much larger number of germs than the water filtered through deep shore sand, and this may have been the cause of the larvæ grown in it being only .7% larger than the normal, as compared with the increase of 8.5% produced by the other water.

After the Aquarium tank sand filter had been kept in darkness for 24 days, and was therefore no longer impregnated with chlorophyll containing growth, another series of determinations was made.

Days sand had been kept in darkness	Time of filtration of 1 litre	Water before filtration 48 hours	Water after filtration 48 hours
24	2h 50′	3700	5300
28	10′	330	150
30	12′	1350	670
30	3h 0′	780	8500

From this table it may be seen that in the two determinations in which the water passed very slowly through the filter, the number of bacteria in the outflowing water was considerably increased. On the other hand, when the rate of flow was moderately quick, the number of germs was diminished to about half. This is rather an unexpected result, for sand filters as a rule remove a greater number of bacteria, the slower the current of water. In this case, however, it is evident that the sand must have contained numbers of multiplying bacteria, so that whilst on the one hand many of the bacteria already in the water would be removed by mechanical filtration, others in larger or smaller numbers would be added to it from the sand itself. It should be mentioned that when determining the number of bacteria in the unfiltered water, the water was drawn off from the tap at approximately the same rate as that at which it had been running through the filter.

The layers of sand used in these experiments were therefore much too thin to act as efficient bacterial filters. Thus, as previously mentioned, they were only 16 cm. thick, instead of the 100 cm. or so usually employed for filtration at water works.

As the following results show, the introduction of algæ into the water seemed to favour bacterial growth.

Water without alga	24 hours	48 hours	Water with alga	24 hours	48 hours
Normal water	510	liquefied	+ 15 sq. cm. *Ulva* per l. for 4 days	1870	liquefied
Normal water + larvæ after 8 days' growth .	1040	—	+ 6 sq. cm. *Ulva* per l. during development .	1550	—
Normal water	3340	7160	+ .6 gm. *Gelidium* per l. for 4 days	22000	liquefied
Normal water	800	850	Water kept 23 days in diffuse light	145	870
Normal water + larvæ after 4 days' growth .	2450	23000	Same water + larvæ after 4 days' growth .	9600	20900
Normal water + larvæ after 8 days' growth .	650	1670	Same water + larvæ after 8 days' growth .	1090	1640

Thus in each of the three instances given, in which some alga was added to the water alone, or to the water containing developing larvæ, the number of bacteria was increased. The next three determinations show the effect of keeping water in diffuse light, whereby a layer of diatoms was formed on the sides of the vessel. The number of germs appeared to be practically the same as in the normal water, and to increase to about the same extent when larvæ were introduced.

It may have been noticed that in all the above results the number of bacteria in the water was invariably larger after two or four days' larval development than after eight days', or than in the water itself before the addition of the fertilised ova. The numbers present after eight days are much more constant than those found at any other period, but they are generally somewhat larger than those in the original samples of water. Thus of the twenty countings made after eight days' larval development, in twelve cases the number of germs after 24 hours' incubation varied between 1000 and 2000, and in only three cases did it rise above 2000. These results are in agreement with those of Bolton, Cramer, Fränkel and others[1], who found that as a rule, owing to the sudden change of temperature and other conditions, the number of bacteria in a specimen of water taken from a river or other source increases very largely for the first few days, and then decreases again.

[1] Untersuchung des Wassers. pag. 481—490.

Part 2. The Fouling Effects of Animal Life on the Water.

The Effects of Animals in General.

Thus far the purification of the water by various forms of vegetable life has been the subject of discussion. We now enter upon the other side of the question, the fouling of the water, especially with reference to animal life. Thus the contamination from vegetable sources seems, in the case of sea-water at least, to be in comparison only slight and unimportant, and those cases in which it does enter in have already been referred to in the first portion of this paper.

In order to estimate the fouling effect of an animal, several individuals were placed for a known time in a known volume of water, and were then removed and weighed. In a portion of the contaminated water larvæ were grown, whilst another portion was subjected to chemical analysis. In the subjoined table the results obtained are given. Here it will be seen that, in addition to the absolute variation produced in the size of the larvæ, the values for the effect produced by 100 grams of the animal kept for one hour in one litre of water have also been calculated. By this means the results obtained with different animals under different conditions are directly comparable. Similarly also the amounts of free and organic ammonia added to the water by keeping 100 grams of the animal in one litre for one hour are given as well as the absolute composition of the water.

Number of Expt.	Animals used	Weight in grams	Time in hours	Volume of water in lit.	% Larvæ formed	% Variat. in size of larvæ	% Variation per 100 gm. p. litre p. hour	Composition of fouled water: free NH_3	Composition of fouled water: organ. NH_3	Var., per 100 gm. per litre per hour in amount of: free NH_3	Var., per 100 gm. per litre per hour in amount of: organic NH_3
45	1 Fish (*Scorpaena ustulata*)	57	3 1/4	2 1/2	85	+1.4	+ 1.8	.377	.090	+.228	— .028
53	2 Fish (*Balistes capriscus* and *Julis vulgaris*	130	1 1/4	2 1/2	17	+8.3	+12.8	.739	1.190	+.725	+1.577
51	4 Crabs (*Pachygrapsus marmoratus*)	61	3	2 1/2	37	+1.6	+ 2.1	.854	.676	+.801	+ .698
66	30 Molluscs (*Cerithium vulgatum, Fasciolaria liquaria, Trochus turbinatus*)	144	2 1/4	1 1/2	57	+2.8	+ 1.3	.514	.382	+.134	+ .094
112	48 Molluscs (*Cerithium vulgatum*) .	240	3 3/4	2	100	+4.8	+ 1.1	.270	.199	+.004	+ .016
49	3 Holothurians (*Holothuria tubulosa*)	317	2 3/4	3 1/2	58	+5.0	+ 2.0	.426	.242	+.063	+ .031
50	3 Holothurians (same) + 6 cm. *Ulva* per l.	-	-	-	59	— .4	— .1	-	-	-	-
71	3 Holothurians (same species) . .	443	3	2 1/2	45	+4.7	+ .9	.483	.262	+.039	+ .022
44	1 Crab + 3 Anemones (*Pagurus & Adamsia*)	122	3 1/4	2 1/2	60	—1.5	— 1.0	1.062	.245	+.538	+ .084
111	3 Anemones (*Adamsia rondeletii*) .	60	3 3/4	2	100	— .6	— .5	.377	.234	+.112	+ .093
72	1 Medusa (*Rhizostoma pulmo*) . . .	66	4 1/4	2 1/2	83	—2.2	— 1.9	.418	.284	+.125	+ .126

Of the eleven experiments recorded in this table, we see that in seven of them an increase in the size of the larvæ was produced. This increase varies in amount from 1.4% to 8.3%, it being on an average 4.1%. Of the other four experiments, one, No. 50, does not properly belong to the present series of experiments, as the effect produced was due to an additional factor, the introduction of seaweed into the water during larval development. In the remaining three experiments, the decrease in size is on an average only 1.4%. This most curious and unlooked for result becomes even more accentuated by a study of the individual experiments. These show that it can be no mere chance whether the water fouled by any particular animal shall have a favourable influence on larval growth or not. As far as the observations go, it would seem a general rule that the excretory products of fish, crabs, molluscs and holothurians have a favourable influence on the growth of larvæ, whilst those of sea-anemones and medusæ on the other hand have a slightly unfavourable one. From the values calculated per unit volume of water, we find that as an average of the two experiments made with fish, the increase is 7.3%. In the single experiment with crabs, the increase is 2.1%: in the two with holothurians it is 1.4%, and in the two with molluscs, 1.2%. Of the three experiments in which a negative effect was produced, a hermit crab with three anemones attached to its shell was used in one case; other anemones but without any crab in the second; and a medusa in the third. The average relative diminution in size is 1.1%.

Upon the percentage number of fertilised ova reaching larval development, fouling of the water does not seem to produce a favourable influence in the same way as it does upon the size, but the effect is in any case only very slightly adverse. Thus as a mean of the eleven experiments, 63.7% of the ova became full grown plutei. Curiously enough it would seem that in those experiments in which there is a negative effect on the size of the larvæ, there is a positive one on the number, and vice-versa, but want of sufficient data must preclude one from drawing any certain conclusion to this effect.

In the last two columns of the table are given the relative amounts of free and organic ammonia added to the water by the fouling. These may probably be taken to give a fair criterion of the relative capacities for fouling which the different animals possess. As might be expected, the numbers are very variable. The relative amounts of free and organic ammonia added by the fish to the water

are on an average respectively .476 and .774 mgm.: by the crabs .801 and .698 mgm., by the molluscs .069 and .055 mgm., and by the holothurians .051 and .026 mgm. Thus the favourable effect produced on the size of the larvæ seems to have been more or less proportionate to the degree of contamination of the water. This is especially borne out by the two experiments with fish, in one of which both the relative increase in the size of the larvæ and the amount of fouling of the water were so very much larger than in the other. Doubtless there is a limit to the favourable effect of the products of animal excretion on larval growth, but apparently it was not reached in any of the above experiments. With regard to the three remaining experiments, in which a negative effect was produced, the influence does not seem proportional to the amount of fouling, but the data are obviously insufficient to decide the question either one way or the other.

These values for the relative amounts of ammonia added to the water have an interest quite apart from their relation to larval growth. Thus they give one an idea as to which of the animals commonly kept in marine aquaria exert the most contaminating influence on the circulating water. These data give no absolute estimate of this influence, but they are quite sufficient to indicate that fish and crabs act very much more harmfully than molluscs and holothurians. Anemones and medusæ apparently occupy a more or less intermediate position, but the fouling effects of the latter animals, considering the large proportion of water in their tissues, is in reality quite considerable.

If an attempt be made to explain these remarkable effects of contaminated water on larval growth, attention should first be directed to some observations cited in the previously mentioned paper[1]. In this it was shown that the addition of one part of uric acid in 70,400 to the water caused an increase of 12.2% in the size of the larvæ, and that it was only when the amount added was increased to one part in 28,000 that an adverse effect was produced. Similarly also the addition of urea to the water increased the size of the larvæ by about 2.7%, and the addition of even considerable quantities of carbonic acid gas also caused a very slight increase. No explanation of these unexpected results was arrived at, but it was suggested that the ideas hitherto entertained as to the adverse effects of products of metabolism on the growth of tissues might be mistaken ones, and

[1] ibid. pag. 595—598.

that in moderate doses these products actually had a stimulating influence, so as to bring about by reaction increased anabolic or constructive tissue changes. The results obtained in this paper seem to support this theory, but it is possible that they and the former results may be due to entirely different causes. Thus we have seen reason to think that bacteria play an important part in the life of these small larvæ, and it may be only through the harmful effect produced on these injurious bacteria that these products of animal excretion are enabled to exert a favourable influence on larval growth. Thus it may be that the larvæ are themselves unfavourably influenced by the products of excretion, but influenced to a greater degree in the opposite direction by the removal of harmful bacteria. However, the few bacteriological observations made with contaminated waters do not seem to bear out this supposition. Thus the specimen of water used in Exp. 112, which was fouled by molluscs, gave 2530 colonies per c.c. after 24 hours incubation, whilst the water used in Exp. 111, which was fouled by sea-anemones, gave 2440. The normal unfouled water gave 2000 colonies. In none of these cases could the colonies be counted after 48 hours, owing to the onset of liquefaction. After eight days larval growth in these waters, the normal water gave 1040 colonies, and that fouled by anemones 1420. The culture of water fouled by molluscs had liquefied. Thus we see that in every case the number of bacteria was slightly greater in the fouled than in the normal water. A similar result, which will be referred to later on, was also obtained with water fouled by sea-urchins.

Though this evidence, and most of that obtained by other investigators into the question of river pollution, tend to show that waters contaminated by animal excretions contain more bacteria than uncontaminated, yet it is still possible to hold that the fouling of a water may happen to kill off certain bacteria which are particularly harmful to larval growth, and that therefore carbonic acid, uric acid, urea, and other products of excretion, act only indirectly. It must be admitted, however, that a direct effect on the larvæ seems much more probable.

The Effects of Echinoids.

We have seem that water fouled by certain fish, crabs, molluscs and holothurians exerts a favourable influence on larval growth, but how do these larvæ react to members of their own and other species of the sea-urchin family? The results obtained on this point are given in the accompanying table.

Number of Expt.	Animals used	Weight in grams	Time in hours	Volume of water in litres	Larvæ used	% Larvæ formed	% Variation in size of larvæ	% Variation per 100 gm. per litre per hour	Composition of fouled water after larval development		Variation, per 100 gm. per litre per hour in amount of	
									free NH_3	organic NH_3	free NH_3	organic NH_3
5	3 *Strongylocentrotus*	106	$3^1/_4$	$2^1/_2$	*Strongylocentrotus*	56	— 4.0	— 2.9	.726	.154	+ .067	— .045
8	5 -	186	$2^1/_2$	4	-	20	— 3.5	— 3.0	.573	.140	+ .003	— .010
19	7 -	402	3	$3^1/_2$	-	72	— 15.0	— 4.4	—	—	—	—
4	1 *Sphærechinus*	362	$3^1/_4$	$2^3/_4$	-	99	— 2.6	— .6	.819	.324	+ .043	+ .002
7	2 -	659	$2^1/_2$	4	-	22	— 6.0	— 1.5	.778	.157	+ .050	+ .002
18	45 *Echinus microtuberculatus*	222	3	$3^1/_2$	-	49	— 10.3	— 5.4	—	—	—	—
46	4 *Arbacia pustulosa*	176	$3^1/_4$	$2^1/_2$	-	81	+ 4.3	+ 1.9	(.514)	(.165)	+ .134	+ .024
48	7 *Dorocidaris papillata* . . .	187	3	$2^1/_2$	-	50	+ 1.7	+ .8	(.492)	(.223)	+ .100	+ .026
12	2 *Sphærechinus*	659	$2^1/_2$	4	*Sphærechinus*	7	— .8	— .2	—	—	—	—
13	5 *Strongylocentrotus*	186	$2^1/_2$	4	-	22	— .2	— .1	—	—	—	—
21	45 *Echinus microtuberculatus*	222	3	$3^1/_2$	*Echinus*	48	— 4.5	— 2.3	.823	.178	+ .176	— .008
22	7 *Strongylocentrotus*	402	3	$3^1/_2$	-	78	— 6.2	— 1.8	.813	.164	+ .094	— .009

In this table the arrangement is the same as in the preceding one, except that as a rule the composition of the fouled water was determined after the larvæ had grown eight days in it, and not before the addition of the fertilised ova. In Expts. 46 and 48, however, the fouled water itself was analysed.

Of the first eight experiments in this table, in which as usual the ova of *Strongylocentrotrus* were employed, we see that in only two cases was a positive effect produced on the larvæ. In every experiment with water fouled by the sea-urchins *Strongylocentrotus*, *Sphærechinus* or *Echinus*, a pronounced diminution in the size of the larvæ resulted. This diminution varies from 2.6 to 15.0%, it being on an average 6.9%. On the other hand a positive effect is produced on larval growth by the excretory products of *Arbacia* and *Dorocidaris*. It would seem as if the negative or positive effect produced by the products of excretion depended more or less on the morphological relationship of the fouling animal. Thus on an average in the three experiments with the water fouled by *Strongylocentrotus*, the effect produced, calculated per 100 grams weight of animal per litre per hour, is 3.4%. On the other hand in the two experiments with the water fouled by *Sphærechinus*, the average diminution is only 1.05%.

In order to obtain further evidence as to the special fouling effect of an animal on its own larvæ, a few experiments were made with other plutei, namely those of *Sphærechinus* and *Echinus*. These were killed and preserved after eight days' growth, in the same way as the *Strongylocentrotus* larvæ. In Expts. 12 and 13, with *Sphærechinus* larvæ, very little effect was produced, but such as it is, it lends support to the other results obtained. In Expts. 21 and 22, the plutei of *Echinus* were used. Here also a more adverse effect is produced by the excretory products of *Echinus*, than by those of *Strongylocentrotus*. The reciprocal experiment, with *Strongylocentrotus* plutei, gave, on the other hand, a contrary result. Thus in Exp. 18, with water fouled by *Echinus*, the diminution is 5.4%, whilst in Exp. 19, with water fouled by *Strongylocentrotus*, it is only 1.4%. This single contrary result cannot be considered to balance the very positive ones obtained with *Strongylocentrotus* and *Sphærechinus*, and hence the existence of a special fouling effect of an organism upon members of its own species may be recognised as highly probable. A comparison of the numbers of ova reaching the larval stage in the various experiments is also in favour of this view. Thus of the

Strongylocentrotus ova grown in water fouled by *Strongylocentrotus*, on an average 49% developed, whilst with the water fouled by *Sphærechinus*, 61% developed. Again in the experiments on *Sphærechinus* larvæ, only a third as many of the ova developed in water fouled by *Sphærechinus*, as in that fouled by *Strongylocentrotus*. Still again, in the experiments with *Echinus* larvæ, only 48% of the ova developed in water fouled by *Echinus*, as against 78% in that fouled by *Strongylocentrotus*.

Concerning the actual amount of fouling of the water by these various species of sea-urchins, it is not possible to speak very definitely, as in the first place, chemical analyses were not made at all in several instances, and in the second place the analyses themselves were carried out after and not before the larvæ had been allowed to develop in the water, when of course many of the excreted products may have been absorbed or changed by larval or bacterial growth. That such a change did take place is almost proved by the organic ammonia values. Thus in all the six analyses made, the organic ammonia was found to be either less than, or practically the same as, that present in the normal unfouled water after eight days' larval growth. In the above series of experiments with other animals, we saw on the other hand that the fouled water always, with a single exception, contained very much more organic ammonia than the unfouled. Also in the two experiments made with sea-urchins (Nos. 46 and 48), in which the fouled water was analysed before and not after larval growth, the organic ammonia was appreciably increased. It would seem therefore that the larvæ are in some way or other able to absorb or transmute the organic ammonia introduced into the water by fouling. The free ammonia they do not seem to have much influence upon, for the fouled water, after larval growth, contained as great an excess of it as was generally present in the former experiments in which the fouled water was analysed before the introduction of the larvæ.

What is the chemical nature of these excretory products, which, according to the species of the animal from which they are expelled, will produce either a positive or a negative effect on larval growth? Doubtless comparatively simple nitrogenous bodies as uric acid and urea have a small influence, but as has already been shown, this influence is always a positive one. The major portion of the effect must, one would think, be due to more complicated organic nitrogen derivatives. It was thought that bodies such as the ptomaines might

be one of the important factors, and hence a few experiments were made upon the effects of water fouled by dead sea-urchins, in order to test this supposition. In that the fouling effected by a decomposing organism is so very much greater than that of a living one, the water was exposed to the contaminating agent for only a very few minutes. The results obtained are given in the accompanying table.

Number of Expt.	Animals used	Weight in grams	Time in minutes	Volume of water in litres	% Larvæ formed	% Variation in size of larvæ	% Variation per 100 gm. per litre per hour	Composition of fouled water		Variation, per 100 gm. per litre per hour in amount of	
								free NH_3	organ. NH_3	free NH_3	organic NH_3
52	1 *Sphærechinus* . .	300	3	3	100	+ 2.4	+ 48.4	.476	.370	+ 4.16	+ 4.10
135	2 *Strongylocentrotus*	64	10	2½	97	— 1.2	— 27.4	.404	.203	+ 3.80	+ 1.08
136	2 -	—	—	6	77	+ 1.6	+ 89.2	—	—	-	-
137	1 *Sphærechinus* . .	109	10	2½	87	— 5.8	— 80.5	.292	.226	+ .688	+ .950
138	1 - . .	—	—	6	68	— 2.5	— 83.0	—	—	-	-

Here we see that in two out of the five experiments a positive effect, amounting on an average to 2.0%, was produced, and in the remaining three, a negative effect averaging 3.2%. In the first experiment, made in warm weather with a rapidly decomposing sea-urchin, a positive influence was exerted, in spite of the considerable contamination of the water which the chemical analysis proved. In one of the last two experiments, in which the water was fouled by a *Sphærechinus* killed three days previously by a short immersion in fresh water, some of the fouled water thus obtained was diluted with normal sea-water. Both with the diluted and the undiluted water, a considerable reduction in the size of the larvæ was brought about, this reduction being proportional to the amount of products of putrefaction present. In the other pair of experiments, made with two *Strongylocentrotus* individuals, also killed three days previously, the slight negative effect produced by the concentrated fouled water was converted into a positive one on dilution. On the whole therefore it would seem that, in small quantities, even the products of putrefaction of an organism may have a favourable effect on larval growth. The fact that on an average no less than 86% of the fertilised ova used in these experiments reached full larval development, supports this view. It should, however, be pointed out

that this percentage is probably higher than would generally be found to occur, in that four of these experiments happened to be made with ova in such good condition that of those which developed under normal circumstances, no less than 97% reached the larval stage.

That this effect on larval growth is a direct one, and is not due to an indirect influence on bacterial growth, seems probable from the fact that the water used in Exp. 137, which was fouled by a *Spærechinus*, gave respectively 6600 and 10,900 colonies per c.c. after 24 and 48 hours incubation, whilst that used in Exp. 135 gave 7000 colonies after 24 hours growth, and had liquefied after 48 hours. The normal unfouled water gave respectively 3340 and 7160 colonies, or only about half the number.

Chemical analysis showed the fouled water to contain about the same excess of ammonia as when living animals were used. When, however, the values are calculated out for unit weight of animal per litre of water per hour, the enormous contaminating influence of dead organic matter, as compared with that of the living, is rendered apparent. Thus on an average of the three analyses, 2.88 mgm. of free, and 2.04 mgm. of organic ammonia were added to the water, whilst in the twelve analyses of water fouled by living animals, the average additions were respectively only .250 and .230 mgm., or about a tenth as much.

The Effects of Plutei.

In the paper already referred to, mention is made of two experiments in which the larvæ were grown in water in which another batch of larvæ had already developed. A diminution in size was thus effected, due presumably to the influence of the products of larval metabolism. It was determined in the present instance to repeat and extend these observations, especially in order to determine if larvæ were more affected by their own excretory products than by those of other species, after the manner of their reaction to the excretions of the adult organisms. The experiments made are given in the accompanying table.

Number of Expt.	Larvæ previously developing in 10 c.c. of water	Nature of larvæ	% Larvæ formed	% Variation in size of larvæ	Composition of fouled water		Excess, over that present in normal water, of	
					free NH_3	organ. NH_3	free NH_3	organic NH_3
10	90 *Strongylocentrotus* larvæ 9 days	*Strongylocentrotus*	24	— 6.9	.634	.316	.449	.139
16	80 - - 12 -	-	77	— 2.3	.570	.152	.355	.028
17	30 *Sphærechinus* - 12 -	-	65	— 7.8	.900	.180	.685	.056
32	65 *Strongylocentrotus* - 8 -	-	39	— 4.5	.396	.247	.194	.090
33	154 *Echinus* - 8 -	-	52	— 3.3	.413	.139	.211	— .018
35	65 *Strongylocentrotus* - 8 -	*Echinus*	8.5	— 5.7	.396	.247	.194	.090
29	60 - - 9 -	-	2.4	—13.3	.391	.123	.156	— .031
30	48 *Echinus* - 9 -	-	2.7	—11.6	.488	.194	.253	.040

In the eight experiments here given, the larvæ on an average were diminished 6.9% in size. Probably the unfavourable influence would have been still more marked, if the water used had not been filtered through asbestos to remove the larvæ in it. Thus it was only subsequent to the time these observations were made that such filtration was found to have so powerful an influence on the physiological qualities of the water. In that the water was always filtered in the same manner, these experiments are, however, strictly comparable amongst themselves. As regards individual experiments, especially with reference to ‚reciprocal' fouling, no complete set of larval measurements was obtained. Thus in Exp. 32, in which the larvæ of *Strongylocentrotus* were grown in water previously fouled by larvæ of the same species, the diminution in size is 4.5%, as against a diminution of 3.3% when some of the same larvæ were grown in water fouled by *Echinus* larvæ. When, on the other hand, *Echinus* larvæ were grown in specimens of the same water, those in the *Strongylocentrotus*-fouled water were diminished by 5.7%, and those in the *Echinus*-fouled water failed to reach the eight days larval stage at all. This result would seem, therefore, to entirely favour the conclusion that the excretory products of an organism are more injurious to itself than to other organisms; but the other observations made do not agree with this view. Thus in Exp. 30, the larvæ of *Echinus*, when grown in *Echinus*-fouled water, are diminished in size by 11.6%, but, grown in *Strongylocentrotus*-fouled water, by 13.3%. The attempt to grow *Strongylocentrotus* larvæ in these specimens of water was in both instances unsuccessful. Again,

in Expts. 16 and 17, *Strongylocentrotus* larvæ grown in *Strongylocentrotus*-fouled water were diminished by 2.3%, but grown in *Sphærechinus*-fouled water, by 7.8%. On the other hand *Echinus* larvæ in *Strongylocentrotus*-fouled water failed to reach the full larval stage at all. Taken as a whole therefore these results cannot be held to decide definitely in either one direction or the other. As they were so variable, and so troublesome to carry out, they were not continued any further. The numbers representing the percentages of ova reaching larval development also fail to point either one way or the other, they being roughly inversely proportional to the diminution effected in the size of the larvæ.

On the right side of the table are given the absolute amounts of ammonia in the fouled water, and also the excess of this ammonia over that present in the water in which the normal larvæ were grown. It need scarcely be said that when *Echinus* larvæ were grown in the fouled water, other *Echinus* larvæ, to serve as the standard of comparison, were at the same time grown in normal tank water. On an average, the composition of the water, as far as the ammonia is concerned, is about the same as that present in the previous experiments on the fouling effects of various animals; but, as might be expected, it is not nearly so variable. It obviously depends closely upon the number of larvæ which had been developing in the water, and the number of days of development.

The Effects of Certain Salts on Larval Growth.

We have seen that thus far no definite clue has been obtained as to the nature of the particular products of excretion which may cause either a positive or a negative effect on larval growth. They were shown not to be simple organic bodies as urea and uric acid. Is it possible that they may be of an even simpler nature, such as ammonia itself, or some of its derivatives as the amines and amido bodies? With reference to these latter bodies, no observations were made, but the results obtained with the simple salt, ammonium chloride, tend to show that ammonia derivatives may be a very important factor in the question. These results were as follow:

Number of Expt.	Weight of NH_4Cl per litre	Result	% Larvæ formed
115	.0258 gm.	Larvæ diminished 7.3% in size	72
127	.0394	- - 19.0% - -	28
—	.1075	59% of blastulæ formed. Larvæ lived 3 days . .	—
—	.3745	37% - - - No larvæ	—
—	.7890	Most of the ova had disintegrated after 24 hours	—

Here we see that with .0394 gm. of ammonium chloride per litre, or one part in 25,400, only 28% of the fertilised ova reached the eight days' larval stage, and were then diminished 19.0% in size! With larger quantities of the salt, not only were no larvæ obtained, but a considerable proportion of the ova failed even to reach the blastula stage. Finally the addition of one part in 1270 of the salt caused the fertilised ova to rapidly disintegrate.

This exceedingly injurious action of ammonium salts is rendered more striking when compared with that of nitrites and nitrates, bodies which, as is well known, are very frequently the products of bacterial oxidation of ammonia, both in the soil, and in water. In the accompanying table the effects produced by the introduction of small quantities of potassium nitrite and nitrate into the water are given.

Number of Expt.	Weight of salt per litre	% Larvæ formed	% Variation in size of larvæ
27	.2020 gm. potassium nitrite	100	— 3.3
105	.2815 gm. - -	79	+ 2.1
139	.3730 gm. - -	79	— 8.9
140	.5615 gm. potassium nitrate	93	— .9
87	.7380 gm. - -	100	+ 3.9
106	1.2085 gm. - -	50	— 1.5

These results are somewhat variable, but they may be taken to show to show that when less than about .3 gram of potassium nitrite or 1 gram of potassium nitrate per litre are added to the water, the effect produced on the larval growth may be nothing at all, or slightly positive. Thus the percentages of ova reaching full larval development seem to favour the existence of a definite positive action, they being respectively 89.5 and 96.5% when the quantities of nitrite

and nitrate are kept below the above mentioned limits. In Exp. 139 on the other hand, when .3730 qm. of nitrite was added, the marked diminution of 8.9% was effected in the size of the larvæ.

These results therefore show that the nitrification of ammonium salts by the action of bacteria has a very favourable effect upon the physiological properties of the water, and also that nitrates are distinctly less injurious to animal life than nitrites. But in that these latter salts only exert such an action when present in considerable quantities, it would seem to be a matter of little moment whether the nitrification proceed its complete course, or stop at the stage of formation of nitrites.

Part 3. Miscellaneous.

The Effects of Aeration on the Purification of Water.

Aeration has commonly been regarded as one of the chief agents in the purification of natural waters such as river and well waters, and it is also generally regarded as one of the most important aids we possess for increasing the purity of aquarium water. Doubtless it is very necessary to aerate the water of an aquarium well, in order to provide sufficient oxygen for the respiration of the animals contained in it, but the actual aeration can in itself have but little of that oxidising influence on organic impurities which is commonly attributed to it. Thus in a former paper[1], where I have given some 120 analyses of the gases in the Aquarium tank water, it may be seen that in no case did the amount of oxygen present in the water sink below 3.28 c.c. per kilogram of water, or below about 57% of the amount present in water fully saturated with air. As there is always, therefore, a considerable proportion of oxygen present in the water, the oxidising effect exerted upon organic impurities will be little greater if the tension of the oxygen is at its maximum value, or only half as much. The aeration also removes small quantities of the excess of carbonic acid gas dissolved in the water, but how small this quantity must be, and what a small influence is thereby exerted, is proved by the following experiment, given in the paper just referred to. Thus analysis showed that the Aquarium tank water contained about 50% more carbonic acid dissolved in it than

[1] Journ. Physiol. Cambridge Vol. 19 1895 p. 70.

open sea-water. Nevertheless, after drawing a rapid current of air through about a litre of this water, for the space of four hours, only 9% of the carbonic acid was driven off. In fact, most of the carbonic acid excreted by the animals into the water enters into unstable combination with the salts present in solution. Hence aeration can do but little to remove it, whilst most of the harmful influence its presence might exert on the animal life is prevented. Perhaps aeration may have a favourable effect in checking the growth of harmful bacteria which multiply only when the oxygen tension of the water is low, but upon this point nothing definite is known. The experiments on the effects of exposure of water to sun and air on the one hand, and to the sun only on the other, tend to prove that just the reverse is the case, and that aeration causes an increased production of harmful bacteria.

The observations made on the effects of aeration of the water on the growth of sea-urchin larvæ bear out our theoretical conclusion. In the first experiment given in the accompanying table, the water was aerated by allowing it to fall as a fine spray through a height of 1.8 metres. The process was repeated twice, but larvæ grown in the water thus aerated proved to be 4.2% smaller than the normal. This was probably due to some unknown error. In the next three experiments, the water used was aerated by shaking it up violently in a large flask from time to time, over periods of several hours. The larvæ are on an average 2.3% larger than the normal; hence it is possible that excessive aeration may have a slightly favourable effect, though if the mean of the four experiments made be taken, the positive influence averages only .65%, a negligeable amount. Also as a mean of the four experiments, 68% of the fertilised ova reached the larval stage, or practically the same as in normal water.

Number of Expt.	Treatment of water	% Variation in amount of		% Larvæ formed	% Variation in size of larvæ
		free NH_3	organic NH_3		
75	Allowed to fall 1.8 metres 3 times	—	—	40	— 4.2
113	Shaken intermittently 4 hours	— 15	— 8	76	— .7
122	- - 6 -	+ 22	+ 24	56	+ 3.4
131	- - 8 -	— 8	— 10	100	+ 4.1

One would expect the ammonia present to be slightly diminished by the aeration, and such proved to be the case in two out of the three experiments. In Exp. 122, on the other hand, it was distinctly increased.

Aeration of the water seemed to affect its bacterial qualities as little as its chemical and physiological, as the following results show.

Nature of water	Bacteria present before aeration		Treatment	Bacteria present after aeration	
	24 hours	48 hours		24 hours	48 hours
Normal tank water	2000	liquefied	Shaken 4 hours	2040	liquefied
- - -	380	6870	- 6 hours	3300	liquefied
- - - after 4 days' larval growth	2450	23000	- 6 hours: after 4 days' larval growth	540000	720000
Normal tank water after 8 days' larval growth	650	1670	Shaken 6 hours: after 8 days' larval growth	330	1150
Normal tank water	3340	7160	Shaken 8 hours	1800	liquefied

Here we see that in one out of the three experiments, the shaken water apparently contained more bacteria than the unshaken; in another it contained less, and in the third about the same. After 4 days' larval growth in the shaken water, the number of bacteria was very much larger than with the unshaken water, but after 8 days' growth, the number had become less. These negative bacteriological results as to the effect of shaking the water are in agreement with those of Tiemann and Gärtner, Leone, Miquel and others[1], in which various river and other fresh waters were used. Thus even fourteen days' continuous shaking of the water seemed to have no appreciable influence.

The Nitrites present in the Water.

In the experiments on the purifying effects of bacteria upon sea-water, the disappearance of the ammonia, especially of the free ammonia, was shown to be a constant attendant on such purification. What are the chemical changes this ammonia undergoes? Is it in turn converted into nitrites and nitrates by the action of nitrifying

[1] Untersuchung des Wassers pag. 536—539.

organisms, or are the changes of same other nature, such as a reducing one? The frequent occurrence of nitrifying organisms in fresh water has been demonstrated by Uffelmann, Adametz, and others, whilst Gayon and Dupetit, and also Heraeus, have shown the existence of denitrifying organisms[1]. In the present instance, it was thought sufficient to determine only the nitrites present in the water, as a fair criterion of the nitrification taking place can thereby be obtained, and moreover their determination is so very much simpler than that of nitrates. The method adopted was that of Preusse and Tiemann[2]. This is a quantitative colorimetric method depending on the yellow colouration, due to the formation of triamido-azobenzol, produced on the addition of metaphenylene-diamine solution to the acidified water. Standard solutions containing 1, 2, 3, 5 and 10 c.c. of a .001% solution of N_2O_3 in 100 c.c. of water, were freshly made up each time, and by comparing various thicknesses of these solutions with the water under examination, by means of the colorimeter previously described, fairly accurate determinations were made. In that the colour of the standard solutions is not arithmetically proportionate to the amount of nitrite present, and in that it increases gradually on keeping, the method can, however, yield only approximate results.

Determinations made at intervals of a week or two during the months of September and October showed the normal Aquarium tank water to contain .054, .076, .121 and .108 mgm. of N_2O_3 per litre, or on an average, .090 parts per million of water. A specimen of sea-water collected 5 km. from the shore contained .022 mgm. Specimens of water kept in darkness for 35 days were found to contain only .013 and .018 mgm.; those kept 46 days .027, .00, and .009 mgm.; and a specimen kept 66 days .00 mgm. Thus it follows that so far from the bacteria oxidising the ammonia present in the water to nitrites, they remove a considerable proportion of those already present. It is of course possible that the oxidation may have progressed as far as the nitrate stage, but if so, the above results show that this process must have been nearly completed after 35 days. That the water may contain nitrifying organisms is shown by the analysis of a specimen of water which had been heated to 100° C. and then kept 46 days in darkness. At the end of this time,

[1] Untersuchung des Wassers p. 497.

[2] Ber. D. Chem. Ges. 11. Bd. 1878 p. 627.

when other but unheated specimens of water contained on an average only .012 mgm. N_2O_3, this specimen contained 1.080 mgm., or ninety times as much.

Vegetable life other than bacterial seems as a rule to slightly increase the proportion of nitrites present. Thus a water which before filtration through sand impregnated with diatoms and algæ contained .054 mgm., after it contained .067 mgm.; another specimen, .100 mgm. before, and .135 mgm. after. Another specimen, which had been filtered very slowly through Aquarium sand (1 litre in 80 minutes), contained .693 mgm. after larvæ had been allowed to grow eight days in it, whilst water filtered rapidly through the same sand (1 litre in 4 minutes), contained only .045 mgm. after the larval growth. The normal larval water contained .100 mgm. Again, a specimen of water kept 35 days in diffuse light, whereby it was subjected to the action of diatoms, contained .108 mgm. of N_2O_3. Still again, water in which .6 gm. per litre of the red weed *Gelidium* had been left five days, contained .360 mgm., and water to which was added .7 gm. per litre of *Gelidium* with founds of *Ulva* attached to it, contained after eight days larval growth .630 mgm., as against the .450 mgm. of N_2O_3 present in normal larval water.

The growth of larvæ in a water seems generally, but not always, to increase the proportion of nitrites. Thus after eight days' growth in normal water, the increase was found in two cases to be from .054 and .076 mgm. to respectively .100 and .495 mgm. This increase probably depends chiefly upon the amount of organic impurity originally present in the water, and is but little affected by the larval metabolism itself. Thus open sea-water, which originally contained .022 mgm. of N_2O_3, after eight days' larval growth contained only .036 mgm. That it is the organic impurity present, rather than the amount of available ammonia, is shown by an experiment in which .0394 gm. of ammonium chloride was added to the water. After eight days' larval growth, this water contained .108 mgm N_2O_3, as against the .100 mgm. present in the normal water. The amount of nitrification varies considerably in different cases, it probably depending upon the number of nitrifying organisms in the water. Thus in Expts. 97 to 104 inclusive, the various specimens of water contained on an average .087 mgm. N_2O_3 after eight days' larval growth. In Expts. 120 to 129 they contained on an average .140 mgm., but in Expts. 130 to 138 no less than .322 mgm. This considerable increase in the amount of nitrites was present in every experiment

but No. 134, the above mentioned one in which open sea-water was used. It would therefore seem that the specimens of water used must have contained some more than usually powerful nitrifying organisms.

Of the other observations made, only a few need be referred to here. Thus in an experiment in which .5615 gm. of potassium nitrate per litre had been added to the water, only .207 mgm. N_2O_3 was present after eight days' larval growth, as against the .450 mgm. present in the normal water. It would therefore seem that the nitrites formed in the water are not due to a reduction of the nitrates which happen to be present, but must be due to processes of oxidation.

The nitrifying process seems unfavourably affected by aerating the water by violent shaking. Thus shaken water was found in two experiments to contain respectively .067 and .243 mgm. N_2O_3 after eight days' larval growth, as against the .101 and .450 mgm. present in normal water.

With respect to the experiments on the fouling of water by other organisms, it would seem that no increase in the nitrites is present either before or after the growth of larvæ. Thus water, fouled by a putrid *Sphærechinus* contained .100 mgm. N_2O_3, and that by *Strongylocentrotus*, also .100 mgm. The normal water before fouling contained .121 mgm., and hence some immediate reduction of nitrites must have taken place. After eight days' larval growth, the *Sphærechinus*-fouled water contained .252 mgm., and the other water .360 mgm., as against the .450 mgm. present in the normal water.

The Arm-Length Measurements.

In addition to measuring the body lengths of the larvæ, a second measurement, that of the aboral or anal arm length, was made as well. By this means it was hoped to derive additional information as to the physiological effects of the various specimens of purified and fouled water. Thus, as has been shown with some detail in the paper already referred to[1], the arm lengths of the larvæ are not by any means always effected to the same extent, or even in the same direction, as the body lengths, on change of environmental conditions. For instance, increased temperature during development causes a diminution in the body length, and an increase in the arm length: dilution of the water, an increased body length, and no effect at all

[1] ibid. pag. 601—612.

on the arm length. It was also shown that, in contradistinction to the body lengths, the arm lengths were greatly affected by the number of larvæ developing together in a given volume of water. In order to get rid of this very variable environmental factor, it was therefore necessary to introduce a correction, whereby every arm length, whatever the number of larvæ which had been growing together, was converted into its theoretical value at infinite larval dilution. The same correction has been introduced in the anal arm length values obtained in the present instance, and these corrected values are given in the last column of the table at the end of the paper. In the last column but one are given the mean uncorrected arm length values. It is to be noted that the actual length of the arm was always calculated as a percentage on the body length of the larva, and hence these mean numbers represent the percentage, and not the absolute arm lengths.

It will be convenient to briefly discuss the arm length data in the same order as was previously observed with those of the body length. In the following table are given the variations produced by the action of algæ and diatoms upon the water. These values are calculated as percentages on the (corrected) arm lengths of the larvæ grown under normal conditions.

Numbers of Expts.	Treatment of water	% Variations in arm length	Mean variation in arm length
40, 39, 9, 93	Water previously exposed to action of *Ulva*	+3.9, +24.7, +24.7, −23.8	+7.4
43, 41, 108, 24, 89, 2	*Ulva* present during larval development	−.3, −.5, −.6, +66.7, −21.8, −3.9	+6.6
94, 95, 96, 132	Water previously exposed to action of *Gelidium* etc.	−27.0, −19.1, ±0.0, +12.3	−8.4
54, 90, 92, 91	*Gelidium* etc. present during development	+6.4, −9.9, +8.1, −5.1	−.1
42, 68, 133	*Gelidium* + *Ulva* present during development	+18.5, −12.2, +12.6	+6.3
55, 63, 65, 99, 100, 123, 124, 125, 126	Water filtered through sand containing vegetable growth	−8.5, −6.5, +.1, −21.4, −25.4, −2.4, −7.7, +24.1, +6.4	−4.6
73, 129	Water previously kept some weeks in diffuse light	+20.6, −11.3	+4.6

It will be seen that in three out of the four experiments in which the water was previously exposed to the purifying action of the alga *Ulva*, an increase in the arm length resulted. In four out of the six experiments in which the larvæ were grown in contact with the alga, practically no effect was produced; but in one of the remaining two experiments a very marked increase in the arm length was present, and in the other a considerable decrease. These great variations must be set down to some unknown causes. The action of the *Ulva* itself would seem to be either nil, or slightly positive. The action of the red weeds would seem to be also very slight, though red weeds in company with green ones perhaps exert a slightly positive effect. It is to be noted, however, that an apparent absence of effect upon the arm length really means that this part of the organism is affected by the environment to the same degree as the body length, whatever that may happen to be; for, as already mentioned, these arm lengths are percentages on the body lengths.

The effect of filtration of water through sand impregnated with vegetable growth would seem to be a distinctly adverse one. Thus in six out of the nine experiments made a diminution resulted; in one there was no change at all, and in only two was there an increase of arm length. The mean diminution is only 4.6%, whilst the mean increase in the body length of the larvæ in these experiments has previously been shown to be 4.2%. It follows, therefore, that the absolute arm lengths of the larvæ were practically unchanged.

The observations on the influence of algæ are therefore somewhat variable and indefinite, but in any case they serve to prove that no marked effect is brought about. The observations on the effects of bacteria are much more definite, as the following table will show.

Numbers of Expts.	Treatment of water	% Variations in arm length	Mean variation in arm length
25, 37, 61, 62	Water previously exposed to sun and air	+47.8, −35.2, +11.1, −41.7	− 4.5
26, 38, 60	Water previously exposed to sun only	−6.8, −18.4, −5.9	− 10.4
78, 79, 82, 83, 118, 116, 119, 128	- - kept some weeks in darkness	+ 26.0, +30.4, −.5, −6.7, +20.9, +27.6, +42.0, −7.7	+ 16.5
77, 98, 121, 85	Water previously filtered through asbestos	+20.2, −11.3, +27.0, −13.6	+ 5.6
15, 57, 102	Water previously heated to 100° C	−24.6, −49.5, −1.7	−25.3
112, 122, 131	- - - - 50°—77°	−1.8, −4.2, −4.3, +6.1	− 1.0

In the four experiments made with water exposed to the sun for four or more days in a covered jar, there is an average diminution of 4.5% in size. As the numbers are very variable, little reliance can be placed on this result. On the other hand, with water exposed to the sun in a flask filled up to the neck, a negative effect was produced in each case, this amounting on an average to 10.4%. In that the body lengths in these particular experiments were found to increase on an average by 16.7%, it follows that there was still an increase of 6.2% in the absolute arm length.

With water purified by being kept in darkness for some weeks, the increase in arm length is very marked, it amounting on an average to 16.5%. The increase in body length in these particular experiments averaged 7.5%, hence the arm tissues have reacted much more than the body tissues to the environmental change. With water filtered through asbestos there is also a slight increase in arm length, though the figures are too variable to place much reliance in. A previous heating of the water has, on the other hand, a totally different influence upon the growth of the arm and of the body tissues Thus we have previously seen that larvæ grown in water heated to 100° have their body lengths increased by 6.9%, and those in water heated to from 50° to 77°, by 5.6%. The arm lengths on the other hand are diminished by no less than 25.3% with water heated to 100°, but are practically uninfluenced by water heated to 77°. This seems at first sight an almost inexplicable result, as indeed to several of the other results arrived at, but probably they may all of them rightly be ascribed to the effects of bacterial growth. Thus in the former paper it was shown that in these larvæ the first stages of body growth are very much more rapid than those of the arm growth, whilst later on the reverse is the case. For instance, at the end of the third day the body has grown to 90.6% of the length it will attain on the eighth day, whilst the anal arm has reached only 63.8%. It follows therefore that if the tissues of these larvæ are affected by bacteria at all, those of the body will be most influenced by the bacterial condition of the water during the first two or three days of development, and those of the arms by its condition on the subsequent days. For instance, in these experiments on the effects of previously heating the water, the water must at first have contained much fewer bacteria than the normal tank water. On the second day, however, it was found that the numbers of bacteria in the heated water were on an average the same as

in the normal, whilst on the eighth day they were considerably in excess.

Thus far we have seen only the effects of purified water on the arm lengths. In the following table are given the effects of fouled water.

Number of Experiments	Water fouled by	% Variations in arm lengths	Mean variation in arm length
45, 53, 51, 66, 112, 49, 71, 44, 111, 72	various animals	+ 1.2, — 16.9, — 20.4, + 6.6, + 40.0, + 8.5, + 15.2, — 12.8, + 45.9, + 27.3	+ 9.5
5, 8, 19, 4, 7, 18, 46, 48	Echinoids	+ 1.9, — 9.9, — 3.4, + 6.7, — 16.8, — 33.5, — 4.0, — 12.1	— 9.4
52, 135, 136, 137, 138	dead Echinoids	+ 2.8, + 17.1, ± 0.0, + 12.8, — 2.1	+ 6.1
10, 16, 17, 32, 33	Plutei	— 26.0, — 17.1, — 47.0, + 33.0, + 48.2	— 1.8
115, 127	ammonium chloride	— 14.0, — 34.2	— 24.1
27, 105, 139	potassium nitrite	+ 16.9, + 5.4, — .9	+ 7.1
140, 86, 106	potassium nitrate	— 3.0, — 3.6, + 16.0	+ 3.1
74, 113, 122, 131	Water aerated by shaking	+ 8.1, + 27.2, + 5.0, + 10.6	+ 12.9

In seven out of the ten experiments made with water fouled by various animals, the arm lengths were increased in amount, and in the other three, decreased. On an average this increase amounts to 9.5%, or considerably more than the increase in body length, which in these particular experiments amounted to 2.4%. In the three experiments in which the arm lengths were diminished, the foulness of the water, as judged by the amounts of free and organic ammonia present, was greater than in any of the other instances. Very probably therefore, if the fouling had not been carried to so great a degree, a positive effect would have been produced as in the other experiments. The animals producing this fouling were fish, crabs, and a hermit crab with three anemones. In the eight experiments made with water fouled by Echinoids, an unfavourable effect on the arm length was exerted in every case but one. The average diminution in size was 9.4%, or a similar value, only in the opposite direction, to that obtained with animals other than Echinoids. As we have already seen, Echinoid-fouled water

in most cases produces also a decrease in the body length, this amounting on an average to 4.4%. In the two instances in which the water was fouled by *Arbacia* and by *Dorocidaris*, a positive effect was produced on the body length; but nevertheless, the arm lengths were negatively affected. The results obtained with water fouled by putrid Echinoids are not quite parallel for the arm and body lengths. Thus in only one out of the five experiments made was there a decrease in the arm lengths, whilst in three out of the five there was a decrease of body length. There is in fact an average increase of 6.1% in the arm length, but an average decrease of 1.1% in the body length. Again, the results obtained with Pluteifouled water are also rather irregular. Thus in two out of the five experiments made there is a pronounced positive effect on the arm lengths, so that on an average the diminution is only 1.8%. The body lengths on the other hand were in every case affected unfavourably, the average diminution being 5.0%. As a whole, however, these results show plainly that whatever effect may be produced in the body length by the fouled water, be it either positive or negative, a similar but more marked effect is produced in the arm length.

The few experiments made with salts are also included in this table, and they more or less confirm this conclusion as to similarity of effect. Thus in the experiments made with respectively ammonium chloride, potassium nitrite, and potassium nitrate, the average variation in the length of arm is — 24.1%, + 7.1% and + 3.1%, and in the length of body respectively — 13.1%, — 3.4% and + .5%. The unfavourable effect of the ammonium chloride on both the arm and the body lengths is thus very striking; but nitrites and nitrates, in small quantities, probably have little or no effect on either.

Finally, in the last line of the table, are given the effects produced by aeration of the water. In every case a distinctly favourable effect was produced, this amounting on an average to 12.9%. This is a much greater effect than that produced on the body length, which amounted on an average to only .6%. It is presumably due directly to the increased aeration, as the bacteriological observations made did not show much change in the numbers of germs present.

The Chemical and Physical Properties of Aquarium Water.

In each of the series of experiments made on larval development, the Aquarium water in which the fertilised ova were placed was

examined as to its chemical and physical properties. These observations, together with a few additional ones made from time to time, are collected together in the accompanying table.

Date	Temperature of water	Corrected Specific Gravity at 15.56° C.	Amount, in mgm. per litre, of		% larvæ formed	Date	Temperature of water	Corrected Specific Gravity at 15.56° C.	Amount, in mgm. per litre, of		% larvæ formed
			free NH_3	organ. NH_3					free NH_3	organ. NH_3	
9. IV	14.5°	1.02859				1. VII	21.80°	1.02928	.278	.143	69
10.	13.7°	1.02879	.236	.182	77	9.	22.80°	1.02914	.350	.125	96
22.	14.58°	1.02883	.185	.177	42	17.	22.24°	1.02886	.340	.135	
5. V	16.38°	1.02875	.215	.124	100	11. VIII	22.80°	1.02940	.193	.143	63
14.	15.82°	1.02866	.235	.154	39	16.	23.0°	1.02962	.201	.118	
24.	16.94°	1.02897	.202	.157	35	20.	23.2°	1.02964	.219	.122	75
29.	17.9°	1.02898	.223	.134		30.	23.40°	1.02958	(.146	.114)	76
2. VI	18.9°	1.02883	.208	.111	64	10. IX	22.63°	1.02952	.251	.129	65
11.	19.42°	1.02880	.268	.165	52	27.	22.78°	1.02957	(.074	.094)	67
16.	19.28°	1.02893	.227	.167		11. X	16.47°	1.02930	.242	.157	97
22.	19.20°	1.02894	.224	.178	49	4. XI	14.90°	1.02932	.188	.131	

The specific gravity of the water was taken very carefully by means of a specially made hydrometer, which, by comparison with a direct determination made by means of a specific gravity bottle, was found to give accurate results. The values given in the table are all corrected to a temperature of 15.56° C., or 60° F., in accordance with the tables given by DITTMAR[1]. From these corrected values we see that the salinity was at its minimum in the spring. After a slight rise, it kept fairly constant till the end of June, and then began to rise again. It reached its maximum value on Aug. 20th, but from this point till the beginning of November, or as long as the observations were continued, it still kept at nearly the same high value. The extreme range varied from 1.02859 to 1.02964, which corresponds to a variation of about 3.7 % in the salinity. The salinity of the water was only slightly greater than that of open sea-water, as the following (corrected) values show.

[1] Challenger Reports. Vol. I pag. 70.

Date	Distance from shore	Specific Gravity at 15.56° C.	Date	Distance from shore	Specific Gravity at 15.56° C.
9. IV	2 km.	1.02821	11. V	3 km.	1.02890
10. IV	2 km.	1.02795	8. X	5 km.	1.02877
11. V	1 km.	1.02878	5. XI	15 km.	1.02879
-	2 km.	1.02847	8. XI	10 km.	1.02860

On comparing these figures amongst themselves, it will be seen that there is a slight increase of salinity on passing from the spring to the summer and autumn months.

The values given in the table under the heading of temperature are for the water in the large tank of the Aquarium, of which the capacity is about 70 cubic metres. From these it will be seen that the temperature increased steadily till it reached a maximum at the end of August. From this point it at first slowly, but then very rapidly, declined again. In another paper[1] I have given the daily temperatures of the Aquarium water during the winter months, and from these it appears that a minimum value of about 9° is reached at the beginning of January, and is more or less maintained at that point till the end of February.

From the chemical analyses of the water, it may be seen that the ammonia present is very variable in amount. Thus the free ammonia varies from .185 to .350 mgm. and the organic ammonia from .111 to .182 mgm. As has already been shown, open sea-water contains about .010 mgm. of free, and .070 mgm. of organic ammonia. There appears to be little, if any, relation between the amounts of free and organic ammonia present, or between these amounts and the temperature, or the salinity, of the water. Thus the ammonia, within wide limits, keeps about the same during the whole period of the observations, and such variations as are present are probably only fortuitous, depending on the volumes of fresh sea-water pumped into the Aquarium, and the amount of animal life contained in it. Thus the Aquarium water undergoes almost as great changes of composition from day to day as it does from week to week, or month to month. For instance, five analyses of water taken on consecutive days from one of the underground reservoirs showed respectively

[1] Journ. Physiol. Cambridge Vol. 19 1895 pag. 68.

.145, .142, .173, .222 and .247 mgm. of free ammonia, and .098, .116, .127, .185 and .193 mgm. of organic ammonia to be present.

There seems to be a relation between the amounts of organic ammonia present in the water, and the percentages of fertilised ova, which, on development in this water, reached the eight days' larval stage. Thus in the five experiments in which 52% or less of the ova reached full larval development, the organic ammonia present amounted on an average to .166 mgm., whilst in the remaining eleven experiments, in which 63% and upwards developed, it averaged only .131 mgm. The values for the free ammonia were respectively .221 and .219 mgm., or practically the same.

The two sets of ammonia values in the table which are enclosed in brackets, are for water drawn off through one of the taps, and not taken from the large tank of the Aquarium. The ammonia present is considerably smaller in their case, though of course the specific gravity is unaltered.

Practical Conclusions.

Thus far only the experimental work itself has been described and discussed. What are the practical applications of the conclusions arrived at, especially with regard to the maintenance, in as efficient a manner as possible, of marine aquaria on a large scale? To consider the results in the order already described, we have seen that algæ, both green and red, if they can be kept in a healthy condition, may exert a considerable purifying influence on the water. There is no fear that in a marine aquarium the vegetable life will ever outweigh the animal, and hence as many and as varied weeds as possible may be introduced. Thus the green weed *Caulerpa* has recently been introduced into one of the tanks at the Naples Aquarium, and appears to be living healthily. *Ulva* also keeps in good condition, but red weeds are apparently unable to flourish for any length of time. Probably the water itself is initially too impure for these weeds to have a chance of growing, but if the water could once be obtained in a purer condition, there seems to be no reason why they should not grow in it, and help to maintain the higher standard of purity. As previously mentioned, Warington found that these red weeds thrived best when placed in a very dull light, and the few observations made on the subject confirmed this conclusion. Thus several species of red weed, introduced into a very dimly illuminated

tank, were found to be in a perfectly healthy condition six weeks later, whilst such weeds when introduced into brightly illuminated tanks with white marble floors began to decay within a week or two.

Probably a more potent factor than the macroscopic algæ, both for good and for evil, is to be found in the Diatomaceæ and the microscopic algæ. Thus we have seen how rapidly sand impregnated with this vegetable growth can remove the ammonia from the water, but we also saw that if the circulation of the water were diminished to at all a slow rate, it immediately became contaminated with more ammonia than it originally contained. This lowly organised vegetable growth appears to be exceedingly sensitive to the least defect of the water which brings it its food material, and hence it follows that the vegetable slime which becomes deposited in the tanks of aquaria rapidly becomes a contaminating agency. A layer of slime so thin that all strata of it get sufficient circulation of water, is a highly efficient purifying agent, but once the lowest stratum is deprived of this water, it begins to decompose. In order to effectually make use of the purifying action of diatoms and algæ, it would seem to be necessary to filter the water, at one part of its circuit, in a continuous stream through a layer of sand impregnated with this vegetable matter. In the Naples Aquarium the water is only pumped for every alternate two hours, and hence, if it were required to purify this water after it had run out of the tanks, it would have to be collected in a subsidiary reservoir, from which it could filter in a continuous stream through a layer of sand. This layer need not be of more than a few centimetres in depth, though it would probably have to be renewed every few weeks owing to the multiplication in its substance of bacteria which are carried away by the water. This increase of bacteria in the water could of course be obviated by subsequently filtering it through a deep sand filter, such as is used in water works. As to the best rate of filtration, the data obtained on the small scale seemed to show the faster the rate the better, and that with a layer of sand about 16 cm. in depth the greatest possible rate, i. e., at 20 to 30 litres per superficial square metre per minute, is the best. Needless to say, a filter of this kind would have to be exposed to daylight.

Should it, on the other hand, be difficult to arrange for the filtration of the water in a continuous stream, or for the sand itself to be exposed to light, a bacterial sand filter could be employed

instead. For this purpose a larger superficial area of sand would be required, and the rate of filtration would have to be slower. In this case also a considerably greater depth of sand would be beneficial. Again, it would be necessary for the water, previous to its passing through this filter, to pass through a smaller subsidiary one of coarse sand, so as to remove the particles of suspended matter in it. Thus this suspended matter, being mostly of a chlorophyll-containing vegetable nature, would, when deposited on the sand in the absence of light, rapidly undergo decomposition. The sand of the subsidiary filter would therefore need to be frequently renewed, but that of the chief filter probably only seldom.

With regard to the utilisation of sunlight as a purifying agent, it would seem to be scarcely worth while to make special arrangements to expose the water to its influence. No doubt some of the bacteria present would be killed, but as we have seen, even several hours' exposure does not effect very much in this way, whilst on subsequently keeping the water in diffuse light, the unharmed germs begin to multiply rapidly, so that the water becomes bacterially less pure than before.

In order to fully utilise the purifying action of the bacteria in the water, it would seem to be necessary to have exceedingly large reservoirs, in which the water could remain for days, and preferably for weeks. Probably this would not be possible in most instances, but in any case it would seem advisable to have the reservoirs as large as possible. Great care should be taken to remove the sediment as frequently as possible, for, as an experiment previously described proved, this deposit soon undergoes decomposition and effects a marked contamination of the water. Supposing the plan were adopted of filtering through sand the water which ran away from the tanks, this source of impurity would of course be largely avoided.

With regard to aeration, we have seen reason to think that the purifying effect of this agency has been much over-rated. It is of course necessary to keep up the percentage of oxygen in the water, but a good deal would be done in this way if algæ could be successfully maintained. There does not appear to be any very simple method of determining the amount of oxygen dissolved in the water; but it would certainly be worth while, should any doubt be felt as to whether the aeration employed in a particular case were more or less than was really necessary, to make daily determinations of the oxygen for a period of some days or weeks, during which the

conditions of aeration were purposely varied. The method employed might be either direct gas analysis, or the simpler method of Schützenberger and Risler[1].

The free and organic ammonia could of course be easily ascertained from time to time, and a very fair criterion of the amount of fouling of the water be thus obtained.

If the water in an Aquarium is but seldom renewed, it would be advisable from time to time to make careful determinations of the specific gravity, and if necessary, diminish the salinity by the addition of fresh water. This could of course be easily done in a large aquarium by allowing fresh water to flow continuously at the required rate into one of the large tanks or reservoirs. A sudden addition of the water necessary to reduce the salinity to the normal might have disastrous consequences. Thus delicate pelagic animals are especially affected by changes of salinity, and probably one of the chief causes of their very short life in aquaria is due to the shock of sudden transference from open sea-water to the denser aquarium water. In the few comparisons made, the salinity of the Naples Aquarium water was found to be on an average about a hundredth part more than that of the open sea-water: an apparently very small amount, but nevertheless enough to cause a difference of about 180 mm. of mercury in osmotic pressure. To reduce this specific gravity to the normal, it would have been necessary to add about 5000 litres of fresh water to the circulating water of the Aquarium, for the total volume of this amounted to about 500 cubic metres.

General Conclusions.

In addition to their practical bearing, the observations and experiments described in this paper may be held to throw some light on the processes of water purification taking place on a vast scale in nature. They may, I think, be taken to show that as far as sea-water is concerned, by far the largest amount of purification is effected through bacterial agency. In that nine tenths and more of the water of the ocean is plunged in darkness too deep for the growth of any chlorophyll containing organism, one must conclude that bacteria and bacteria alone are there the source of purification. Next to bacteria probably come the Diatomaceæ and the minute algæ,

[1] Untersuchung des Wassers pag. 288.

which are suspended in the water, and to a lesser extent deposited on the sand of our sea shores. Owing to the immense quantities in which they occur, and the rapidity with which they can act, they probably exert a very much greater purifying effect than the larger algæ, which can grow only in shallow waters in the neighbourhood of land.

These experimental results also have a bearing on the purification of fresh waters. Probably in their case the action of bacteria is in comparison not so great as for sea-water. Thus it is only seldom that fresh water occurs in lakes at such depths as to be in absolute darkness, and water which has filtered through moderate depths of soil seems to be thereby rendered almost if not quite bacteria free. On the other hand, algæ grow abundantly in almost all rivers, but probably even in them the minute algæ and Diatomaceæ are the more powerful agency. Thus in all but the foulest streams there is a layer of vegetable growth deposited on the stones and grains of sand in the bed of the river, which is continually exerting its purifying action. The rise and fall of the level of the water in rivers causes a continuous circulation of water backwards and forwards through this vegetable layer on the river bed, whereby the purifying action is greatly increased.

This purifying action of algæ, diatoms and bacteria is becoming more and more recognised every day. Thus PETTENKOFER[1] considers that the self-purification of river-water depends more on vegetable life than on any other agency. LÖW[2] also thinks that algæ play a considerable part, and he mentions several instances in which fresh water algæ have been proved to thrive and increase in weight in water containing putrefactive and other organic bodies. To me it seems that vegetable life is practically the only agency in the purification of water. True, the solid matter suspended in the water is removed by mechanical deposition and filtration, but practically nothing but vegetable life can get rid of the dissolved impurities. Aeration and mechanical disturbance of the water can do little or nothing directly. Sunlight indeed kills some of the bacteria, but these would die of themselves without any such agency, once the water became purified through the vegetable life it contained.

As regards the methods employed in this research, it may be

[1] Arch. Hyg. 12. Bd. pag. 269.
[2] Arch. Hyg. 12. Bd. pag. 261.

concluded that none of them singly can be held to afford an exact estimate as to the purity or otherwise of a water. Combined however, the evidence they afford is much more trustworthy. The adoption of a physiological test of the purity of a water is, I believe, a method not hitherto employed. Whether it could be profitably used for testing the purity of fresh waters, is somewhat doubtful. Probably for testing drinking waters it would be of little avail, as all of these should be in such a high state of purity that the slight differences actually occurring in them could effect but little in the rate of growth of an organism. For testing markedly impure waters, however, it might be found a valuable agent. In the present experiments, in which Echinoid Plutei were made use of for the physiological test, it may perhaps be objected that the larvæ were never fed, and hence had to subsist on the animal matter already present in the water, and that therefore, the more of such matter a water contained, the greater ought to be the size of the larvæ. This criticism is probably not valid, as most likely these larvæ do not feed at all up to the eighth day or so of growth, when they were killed and measured, but subsist on the food material originally present in the ovum. Thus Davenport has shown [1] that frogs' embryos, though they increase about sixfold in absolute weight during the first ten days of development, contain at the end of this period less solid material than the original ova. The increase in weight seems to be entirely due to the imbibition of water. Again, the mere fact that the larvæ grown in pure open sea-water were some 16% larger than those grown under similar conditions in Aquarium water, shows there can be little in this argument.

Summary.

The following are the chief conclusions [2] arrived at in this paper:

Green weeds such as *Ulva* rapidly remove the free ammonia from Aquarium tank water, but they slowly increase the organic ammonia. Larvæ grown in water thus purified are as a rule increased in size. When grown in direct contact with the alga, they are generally decreased in size, but an increased proportion of the fertilised ova develop to larvæ.

[1] Proc. Boston Soc. N. H. Vol. 28. 1897 pag. 73.

[2] An abstract of these results has been published in the Proc. R. Soc. London Vol. 63 pag. 155.

Red weeds as *Gelidium* generally cause an increase in the free as well as in the organic ammonia. They sometimes have a favourable, but as a rule an unfavourable, effect on larval growth.

Filtration of water through sand may remove almost all the free ammonia present and a third or more of the organic ammonia, this purification being effected by the layer of diatoms and algæ on the grains of sand. Sand kept in darkness may also effect great purification, in this instance through bacterial influence. Larvæ grown in water thus purified are increased in size some 4.2%.

On keeping water in diffuse light for some weeks, nearly all the free ammonia disappears owing to the multiplication of the small quantities of algæ and diatoms originally present. The organic ammonia is at the same time increased, but larvæ grown in the water are greatly increased in size.

Larvæ grown in water previously exposed under certain conditions to the sun, are greatly increased in size. Sunlight, though it has an immediate germicidal action on the water, probably effects no permanent bacterial purification.

On keeping water in darkness for three or more weeks, nearly all the free, and a third or more of the organic ammonia, are removed by bacterial action. Larvæ grown in water thus purified are some 7.5% larger than the normal. As far as the ammonia is concerned, water kept in darkness may become as pure as open sea-water; but it is not physiologically so pure, for larvæ grown in the latter are 16.0% larger than the normal.

A very considerable purification of the Aquarium water is effected by the layer of bacterial slime coating the inside of the conducting pipes. Thus half of the ammonia was sometimes removed by this agency, and the larvæ grown in the water were 7.8% larger than the normal.

Larvæ grown in water filtered through asbestos, and water previously heated to 50°, 76° or 100°, are considerably increased in size.

Larvæ grown in water previously fouled by fish, crabs, Molluscs and Holothurians, are increased in size, but in water fouled by Echinoids and other Plutei, are considerably diminished. Dead Echinoids foul the water about ten times more than most living animals, whilst fish and crabs foul it ten times more than Molluscs and Holothurians.

Ammonium chloride acts very injuriously on larval growth, but

potassium nitrite and potassium nitrate in small quantities have no action.

Aeration has only a very slightly favourable effect on larval growth.

The nitrites present are greatly diminished on keeping the water in darkness, but are increased by vegetable growth, and on development of larvæ in the water.

The arm lengths are as a rule affected in the same direction as the body lengths on change of environmental conditions, but in the reverse direction by water filtered through sand, water exposed to sunlight and water previously heated to 100° C.

The specific gravity and purity of the Aquarium water vary but little at different times of the year. The salinity is about a hundredth part greater than that of open sea-water.

Number of Expt.	Date of Fertilisation	Number of Echinoids used	Temperature of impregnation	Mean temperature during development	Number of ova per 10 c.c.	% blastulæ formed	% larvæ formed	Nature of water	Mean body length	Mean arm length	Corrected arm length
1	10. IV	1♀ . 1♂	13.7°	15.6°	110	100	77	Normal	30.44	108.98	127.5
2	-	-	-	-	123	73	51	+ 60 sq.cm. of *Ulva* per litre	29.41	108.85	122.5
3	-	-	-	-	87	100	88	Fresh open sea-water	33.38	121.24	140.5
4	-	-	-	-	111	100	99	Water fouled by *Sphærechinus*	29.64	111.57	136.1
5	-	-	-	-	180	76	56	- - - *Strongylocentrotus*	29.05	104.26	125.1
6	22. IV	1♀ . 1♂	15.8°	17.5°	189	80	42	Normal	36.30	116.25	134.9
7	-	-	-	-	125	79	22	Water fouled by *Sphærechinus*	34.13	107.50	112.3
8	-	-	-	-	87	74	20	- - - *Strongylocentrotus*	35.04	117.50	121.5
9	-	-	-	-	120	81	4.2	- in which *Strongylocentrotus* larvæ had developed with alga	36.28	123.11	124.3
10	-	-	-	-	147	66	24	Water in which *Strongylocentrotus* larvæ had developed alone	33.82	93.25	99.8
11*	-	5♀ . 1♂	16.5°	-	170	92	32	Normal *Sphærechinus* fertilisation	19.13	215.35	—
12*	-	-	-	-	93	73	6.5	Water fouled by *Sphærechinus*	18.98	227.50	—
13*	-	-	-	-	158	85	22	- - *Strongylocentrotus*	19.10	212.66	—
14	5. V.	1♀ . 1♂	17.9°	17.4°	60	100	100	Normal	32.18	134.09	154.6
15	-	-	-	-	120	97	4.2	Water previously heated to 100° C.	32.63	115.37	116.6
16	-	-	-	-	98	89	77	- in which *Strongylocentrotus* larvæ had developed	31.43	111.53	128.2
17	-	-	-	-	100	97	65	- - - *Sphærechinus* - - -	29.68	72.59	82.0
18	-	-	-	-	72	83	49	- fouled by *Echinus*	28.88	96.12	102.8
19	-	-	-	-	85	82	72	- - - *Strongylocentrotus*	27.36	133.30	149.3
20*	-	5♀ . 3♂	17.9°	-	56	80	86	Normal *Echinus* fertilisation	32.82	104.34	—
21*	-	-	-	-	52	87	48	Water fouled by *Echinus*	31.36	95.36	—
22*	-	-	-	-	27	78	78	- - - *Strongylocentrotus*	30.78	112.77	—
23	14. V.	1♀ . 1♂	17.9°	17.2°	167	100	39	Normal	31.77	86.16	97.4
24	-	-	-	-	197	100	90	+ 6 sq.cm. of *Ulva* per litre	27.57	119.78	162.3
25	-	-	-	-	309	99	25	Water exposed to sun and air 4 days	31.32	124.65	144.0
26	-	-	-	-	277	99	95	- - - - - alone 4 days	36.41	58.80	90.8
27	-	-	-	-	296	100	100	+ .2020 gm. of potassium nitrite per litre	30.71	71.26	113.9
28*	-	5♀ . 2♂	17.5°	-	220	100	70	Normal *Echinus* fertilisation	30.83	62.59	—
29	-	-	-	-	207	96	2.4	Water in which *Strongylocentrotus* larvæ had developed	26.73	73.40	—
30	-	-	-	-	337	94	2.7	- - - *Echinus* - - -	27.17	88.53	—
31	24. V.	1♀ . 6♂	19.0°	19.0°	170	100	35	Normal	29.74	71.82	80.3
32	-	-	-	-	88	100	39	Water in which *Strongylocentrotus* larvæ had developed	28.41	100.02	106.8
33	-	-	-	-	113	100	52	- - - *Echinus* - - -	28.77	106.63	119.0
34*	-	4♀ . 5♂	18.9°	-	100	92	54	Normal *Echinus* fertilisation	33.64	77.79	—
35*	-	-	-	-	200	96	8.5	Water in which *Strongylocentrotus* larvæ had developed	31.72	57.40	—

Number of Expt.	Date of Fertilisation	Number of Echinoids used	Temperature of impregnation	Mean temperature during development	Number of ova per 10 c.c.	% blastulæ formed	% larvæ formed	Nature of water	Mean body length	Mean arm length	Corrected arm length
36	2. VI	2♀ . 2♂	20.1°	21.0°	85	96	64	Normal	31.25	104.61	116.6
37	-	-	-	-	105	93	6.7	Water exposed 4 days to sun and air	31.45	74.47	75.6
38	-	-	-	-	70	100	83	- - 4 - - - only	36.87	85.31	95.2
39	-	-	-	-	90	100	76	- - 4 - - - + 6 sq.cm. *Ulva* per l.	36.51	128.10	145.4
40	-	-	-	-	120	100	65	- kept 4 days with 12 sq.cm. *Ulva* per litre	35.75	106.50	123.1
41	-	-	-	-	83	100	72	+ 6 sq.cm. *Ulva* per litre	31.11	104.78	117.2
42	-	-	-	-	81	100	99	+ .4 gm. *Gelidium* and 3 sq.cm. *Ulva* per litre	30.97	118.24	137.1
43	-	-	-	-	86	100	100	+ 4.5 sq.cm. *Ulva* per litre	32.51	98.35	116.2
44	-	-	-	-	100	92	60	Water fouled by hermit crab and anemones	30.78	90.75	101.7
45	-	-	-	-	73	99	85	- - - a *Scorpæna*	31.67	104.92	118.0
46	-	-	-	-	103	99	81	- - - *Arbacia*	32.60	96.12	111.9
47	11. VI	2♀ . 4♂	20.5°	20.8°	63	100	52	Normal	29.37	125.36	133.5
48	-	-	-	-	86	100	50	Water fouled by *Dorocidaris*	29.88	108.03	117.3
49	-	-	-	-	40	100	58	- - - Holothurians	30.83	138.45	144.8
50	-	-	-	-	17	100	59	- - - - + 6 sq.cm. *Ulva* per l.	29.26	122.04	124.5
51	-	-	-	-	35	80	37	- - - crabs (*Pachygrapsus*)	29.83	103.43	106.2
52	-	-	-	-	40	100	100	- - - putrid *Sphærechinus*	30.08	125.97	137.2
53	-	-	-	-	47	36	17	- - - fish	31.81	109.33	111.0
54	-	-	-	-	98	97	74	+ .2 gm. *Gelidium* per litre	30.58	124.05	142.1
55	-	-	-	-	30	100	100	Water filtered through 14 cm. coarse Aquarium sand	31.18	113.30	122.1
56	22. VI	2♀ . 2♂	20.6°	21.5°	142	93	49	Normal	29.55	107.25	122.2
57	-	-	-	-	115	98	85	Water previously heated to 100° C.	33.56	51.58	61.7
58	-	-	-	-	88	99	66	- - - - 77° C.	29.57	107.55	120.0
59	-	-	-	-	90	100	100	- - - - 50° C.	32.03	98.58	116.9
60	-	-	-	-	187	96	9.1	- exposed 5 days to sun only	34.68	111.00	115.0
61	-	-	-	-	180	98	6.7	- - 5 - - - and air	33.70	132.52	135.8
62	-	-	-	-	177	99	4.0	- - 5 - - - - - in flat dish	24.22	70.05	71.1
63	-	-	-	-	123	98	100	- filtered through 11 cm. coarse sand	31.06	91.78	114.3
64	-	-	-	-	128	92	80	- - - 7 cm. sand previously heated to 70°	29.92	70.38	84.9
65	-	-	-	-	87	100	98	Water filtered through 11 cm. fine sand	31.15	104.58	122.3
66	-	-	-	-	135	87	57	- fouled by molluscs (*Cerithium*)	30.37	112.88	130.3
67	-	-	-	-	83	93	14	+ a *Cerithium* with 6 sq.cm. *Ulva* attached to shell	29.31	96.78	99.3
68	-	-	-	-	97	98	55	+ .13 gm. *Gelidium* and 6 sq.cm. *Ulva* per litre	30.62	97.14	107.3
69	-	-	-	-	138	96	86	Open sea-water 3 km. from shore	35.22	110.25	136.2

Number of Expt.	Date of Fertilisation	Number of Echinoids used	Temperature of impregnation	Mean temperature during development	Number of ova per 10 c.c.	% blastulæ formed	% larvæ formed	Nature of water	Mean body length	Mean arm length	Corrected arm length
70	1. VII	4 ♀ . 2 ♂	24.4°	23.2°	58	98	69	Normal	29.67	92.18	99.6
71	-	-	-	-	67	100	45	Water fouled by Holothurians	31.06	108.12	114.7
72	-	-	-	-	30	93	83	- - - a Medusa (*Rhizostoma*)	29.03	120.81	126.8
73	-	-	-	-	78	92	35	- kept 33 days in diffuse light	36.04	113.67	119.9
74	-	-	-	-	57	100	40	- aerated by falling 1.8 metres 3 times	28.42	103.87	108.5
75	-	-	-	-	58	98	70	- drawn off from tap	32.88	87.46	94.4
76	9. VII	5 ♀ . 3 ♂	23.3°	24.1°	25	92	96	Normal	24.49	90.17	94.3
77	-	-	-	-	38	100	66	Water filtered through asbestos	30.09	107.98	113.3
78	-	-	-	-	40	88	45	- kept 23 days in semi-darkness	28.84	114.76	118.8
79	-	-	-	-	40	95	88	- - 23 - - absolute darkness	28.75	114.90	123.0
80	-	-	-	-	27	100	67	- drawn off from tap	26.53	85.65	88.7
81	11. VIII	8 ♀ . 1 ♂	23.6°	23.5°	35	100	63	Normal	29.97	110.42	115.4
82	-	-	-	-	62	84	45	Water kept 25 days in darkness	31.24	108.80	114.8
83	-	-	-	-	43	100	70	- - 25 - - - + previously kept water	33.38	101.70	107.7
84	-	-	-	-	84	51	15	- - 25 - - jar containing algæ	31.47	62.64	64.2
85	-	-	-	-	62	77	48	- filtered through asbestos, and kept 25 days in darkness	32.19	89.29	94.7
86	-	-	-	-	68	37	37	Water heated to 100° C. and kept 25 days	28.96	40.39	42.4
87	-	-	-	-	23	100	100	+ .7380 gm. of potassium nitrate per litre	31.15	104.80	111.3
88	20. VIII	8 ♀ . 1 ♂	23.9°	23.2°	72	100	75	Normal	31.09	106.64	118.0
89	-	-	-	-	83	81	69	+ 15 sq.cm. of *Ulva* per litre	30.59	82.72	92.2
90	-	-	-	-	97	96	24	+ .8 gm. of *Gelidium* per litre	29.80	101.68	106.3
91	-	-	-	-	97	96	23	+ .56 gm. of *Dictyota* per litre	30.11	107.09	112.0
92	-	-	-	-	93	78	51	+ 1.2 gm. of *Dudresnaya* per litre	30.26	116.42	127.4
93	-	-	-	-	67	100	100	Water kept 4 days with 12 sq.cm. of *Ulva* per litre	30.78	78.84	89.9
94	-	-	-	-	33	100	91	- - 4 - - 1.0 gm. *Gelidium* - -	29.81	81.36	86.2
95	-	-	-	-	80	96	13	- - 4 - - 2.0 gm. *Dudresnaya* - -	25.47	93.55	95.5
96	-	-	-	-	73	92	92	- - 4 - - .7 gm. *Dictyota*	28.98	103.99	118.0
97	31. VIII	3 ♀ . 3 ♂	22.6°	22.9°	75	100	76	Normal	30.80	98.84	110.1
98	-	-	-	-	73	99	64	Water filtered through asbestos	33.56	89.07	97.6
99	-	-	-	-	112	98	54	- - - 16 cm. Aquarium tank sand	30.97	77.35	86.6
100	-	-	-	-	106	99	8	- - - 16 cm. surface shore sand	32.24	80.79	82.1
101	-	-	-	-	95	100	58	- - - 16 cm. sand from 1 metre beneath surface	31.34	70.28	78.0
102	-	-	-	-	116	98	73	Water previously heated to 100° C.	32.78	92.49	108.2
103	-	-	-	-	59	100	81	- - - - 76° C.	33.26	96.26	105.5
104	-	-	-	-	103	100	66	- - - - 50° C.	32.62	102.75	116.8
105	-	-	-	-	85	100	79	+ .2815 gm. of potassium nitrite per litre	31.44	76.21	86.5
106	-	-	-	-	80	100	50	+ 1.2085 gm. of potassium nitrate per litre	30.34	88.07	95.2

Number of Expt.	Date of Fertilisation	Number of Echinoids used	Temperature of impregnation	Mean temperature during development	Number of ova per 10 c.c.	% blastulæ formed	% larvæ formed	Nature of water	Mean body length	Mean arm length	Corrected arm length
107	10. IX	3♀ . 2♂	22.8°	23.2°	178	100	65	Normal	32.36	70.18	86.2
108	-	-	-	-	123	99	99	+ 6 sq. cm. of *Ulva* per litre	32.14	68.81	85.7
109	-	-	-	-	123	100	41	+ a *Cerithium* with 15 sq. cm. *Ulva* attached to shell	31.19	63.90	70.3
110	-	-	-	-	77	100	42	+ a *Cerithium*	28.28	59.45	63.3
111	-	-	-	-	120	98	100	Water fouled by sea-anemones	32.16	98.72	125.8
112	-	-	-	-	132	97	100	- - - molluscs (*Cerithium*)	33.90	93.21	120.7
113	-	-	-	-	187	100	76	- shaken intermittently 4 hours	32.14	85.34	109.6
114	-	-	-	-	177	97	69	- drawn off from tap	33.71	79.90	99.6
115	-	-	-	-	250	100	72	+ .0258 qm. of ammonium chloride per litre	30.00	54.44	74.1
116	-	-	-	-	240	97	54	Water kept 55 days in darkness	33.34	87.40	110.0
117	-	-	-	-	302	99	62	- filtered through asbestos and kept 55 days in darkness	32.34	97.58	134.2
118	-	-	-	-	253	100	38	Water heated to 100° C. and kept 55 days in darkness	32.40	87.04	104.2
119	-	-	-	-	192	100	27	- kept 55 days in darkness, with previously kept water	34.18	111.81	123.4
120	28. IX	6♀ . 3♂	21.2°	20.0°	45	89	67	Normal	32.25	110.58	117.2
121	-	-	-	-	22	82	45	Water filtered through asbestos	34.18	145.79	148.8
122	-	-	-	-	23	100	56	- shaken intermittently six hours	33.36	120.08	123.1
123	-	-	-	-	18	100	72	- filtered fast through Aquarium sand	32.42	111.63	114.4
124	-	-	-	-	17	88	88	- - slowly through Aquarium sand	32.90	105.04	108.2
125	-	-	-	-	33	91	100	- - through 1 metre deep sand	34.98	134.84	145.5
126	-	-	-	-	17	71	88	- - - same sand without top crust	33.60	120.98	124.7
127	-	-	-	-	18	94	28	+ .0394 gm. of ammonium chloride per litre	26.11	76.17	77.0
128	-	-	-	-	18	100	72	Water kept 27 days in semi-darkness	32.37	105.41	108.2
129	-	-	-	-	17	77	71	- - 27 - - diffuse light	34.00	95.30	97.7
130	12. X	2♀ . 3♂	16.5°	17.3°	160	100	97	Normal	33.38	88.80	116.3
131	-	-	-	-	153	100	100	Water shaken intermittently 8 hours	34.76	95.94	128.6
132	-	-	-	-	175	100	87	- kept 4 days with .6 gm. *Gelidium* per litre	33.38	100.21	130.6
133	-	-	-	-	163	99	79	+ .3 gm. *Gelidium*, with *Ulva* attached, per litre	33.24	104.28	131.0
134	-	-	-	-	210	99	95	Open sea-water (5 km. from shore)	37.63	109.68	153.6
135	-	-	-	-	148	99	97	Water fouled by putrid *Strongylocentrotus*	32.99	106.04	136.2
136	-	-	-	-	207	98	77	Same water diluted	33.91	88.19	116.3
137	-	-	-	-	153	100	87	Water fouled by putrid *Sphærechinus*	31.43	103.69	131.2
138	-	-	-	-	222	98	68	Same water diluted	32.54	87.59	113.9
139	-	-	-	-	140	98	79	+ .3730 gm. potassium nitrite per litre	30.42	94.29	115.3
140	-	-	-	-	212	93	93	+ .5615 gm. potassium nitrate per litre	33.07	80.63	112.8